Photosynthesis

Rashmi MC

Photosynthesis- A process from which plants make food!!!

- A physiological process,
- Photosynthetic organisms use light energy to drive the synthesis of organic molecules
- Light energy → chemical energy
- Chemical energy used to reduce CO_2 into organic compounds (**Carbon**

Photosynthetic organism
(On the basis of generation of **Oxygen** during photosynthesis)

Oxygenic organisms

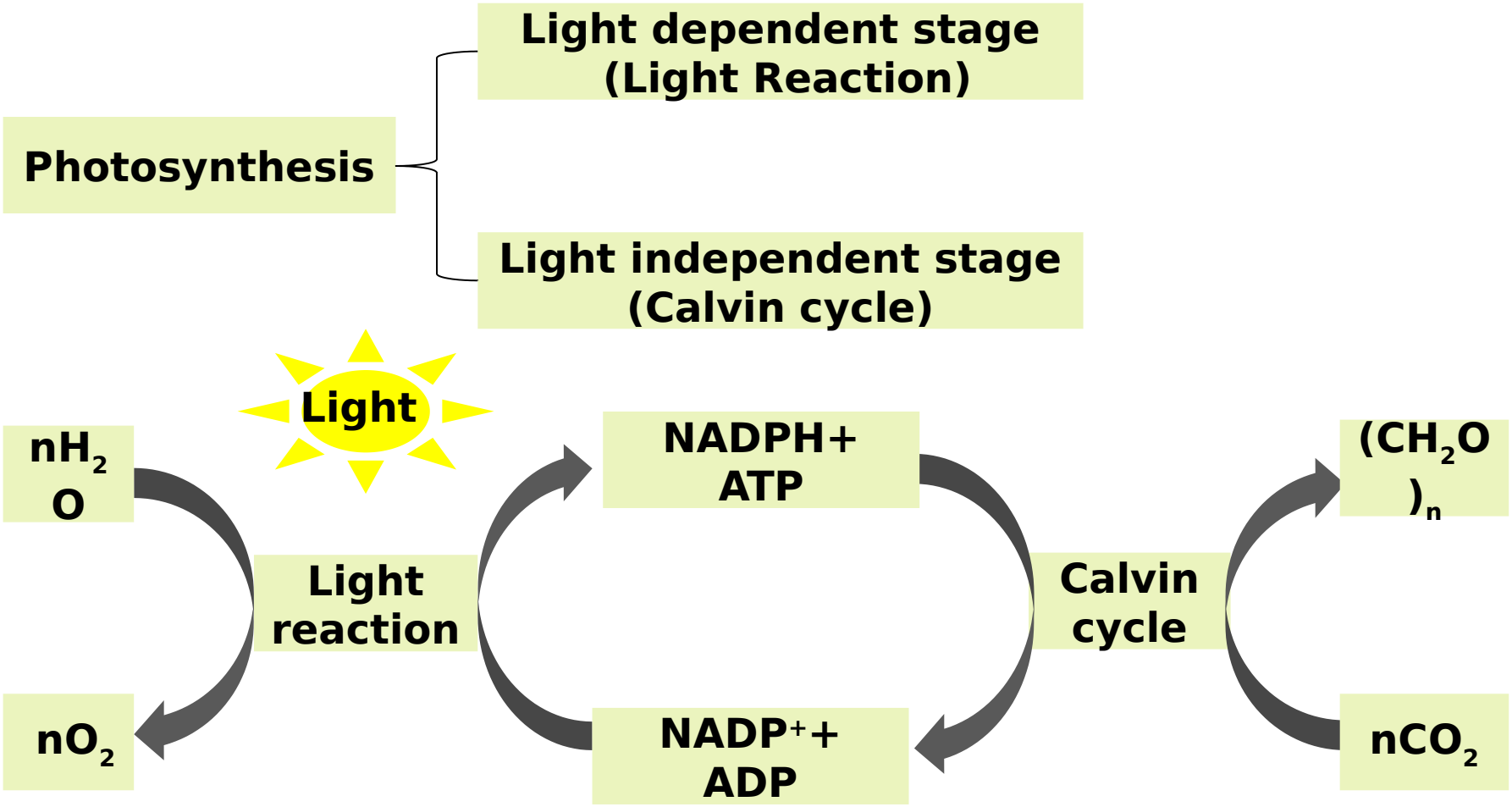
Eukaryotes- Green plants, photosynthetic protists
Prokaryotes- Cyanobacteria

Anoxygenic organisms

Prokaryotes- Green and purple photosynthetic bacteria

Basic reaction of photosynthesis:

$$nCO_2 + nH_2O \xrightarrow[\text{Light, Chlorophyll}]{} (CH_2O)_n + nH_2O + nO_2$$



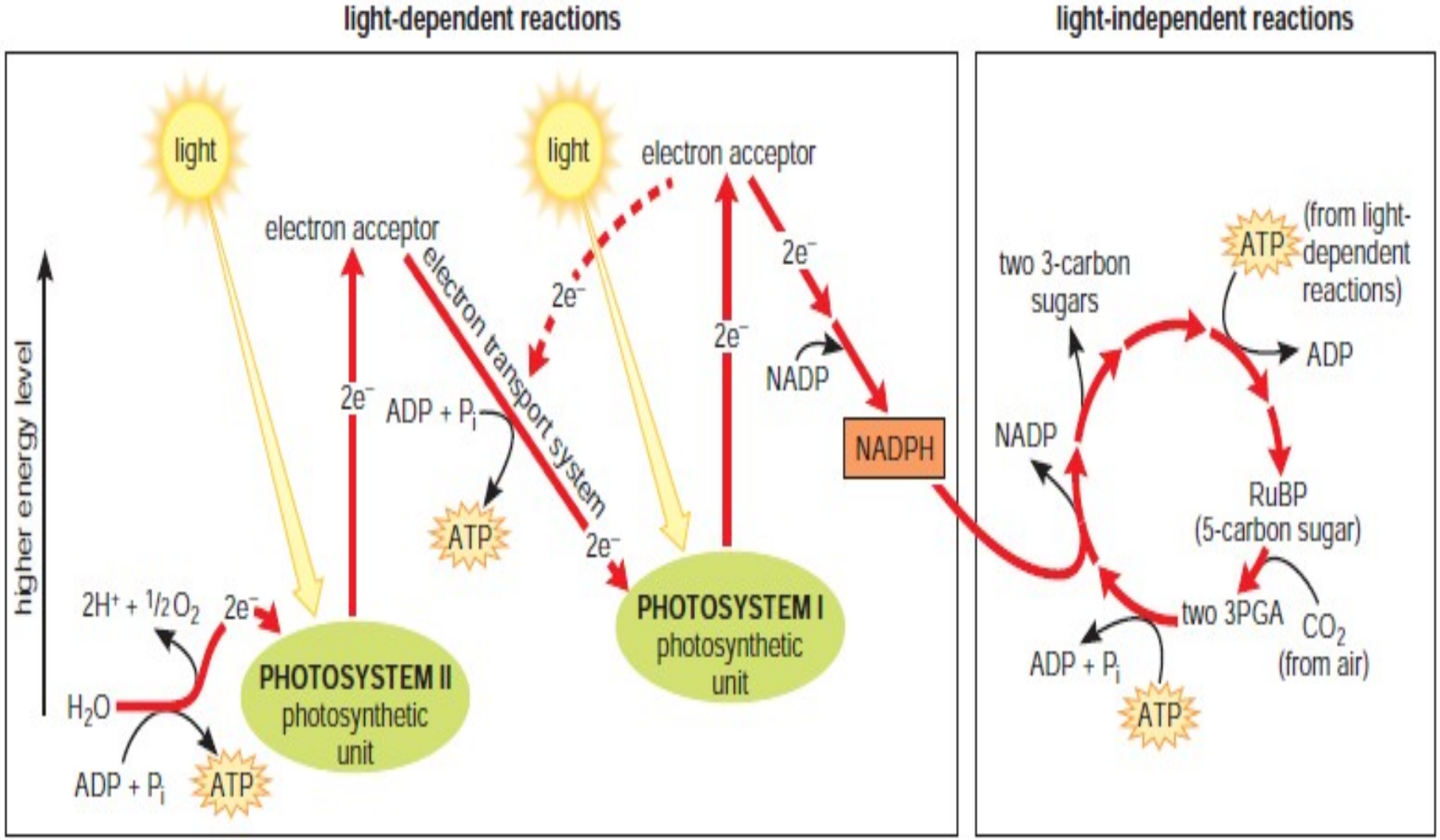


Figure 10.5 A simplified summary of photosynthetic reactions.

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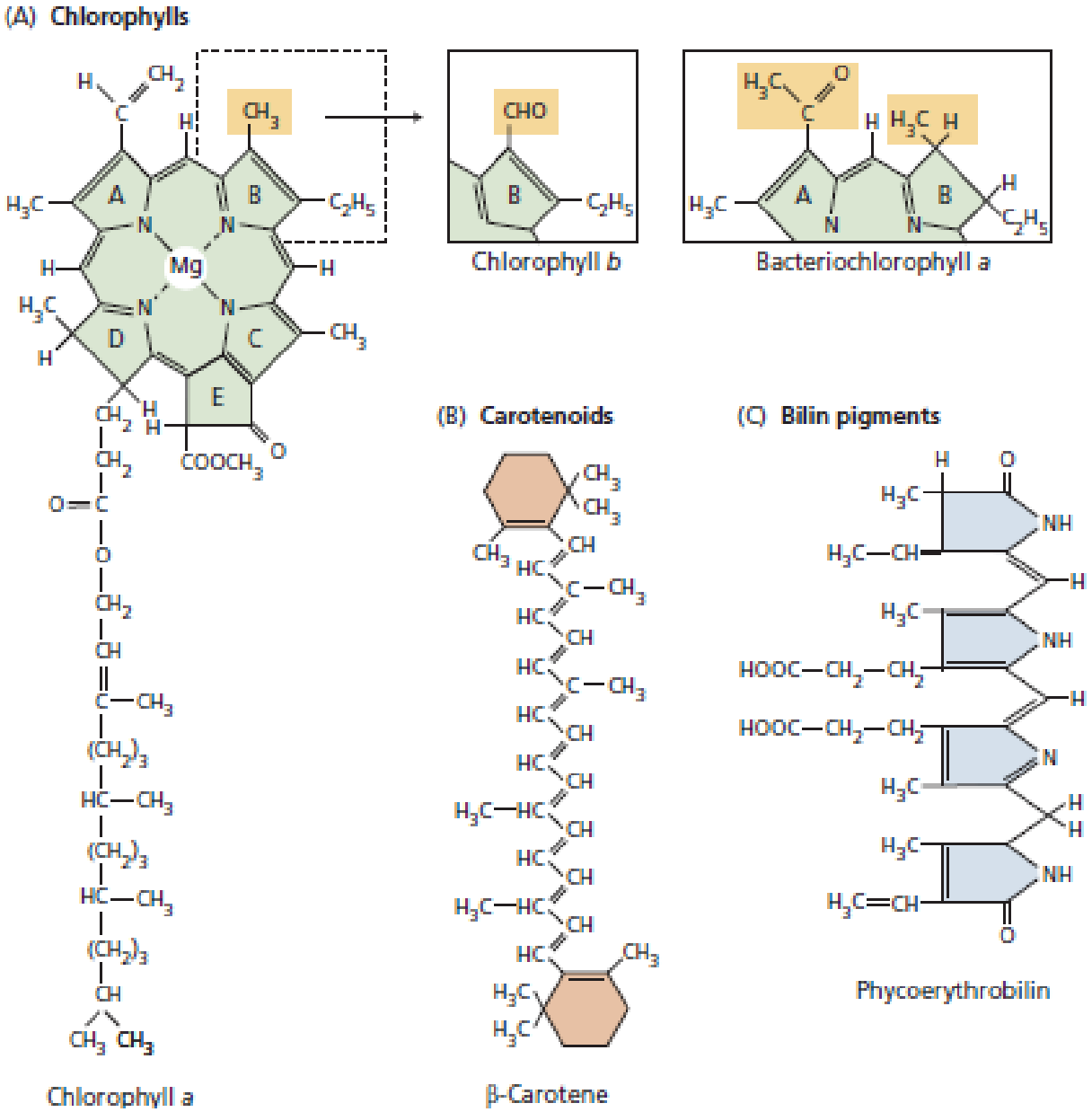
Photosynthetic pigments

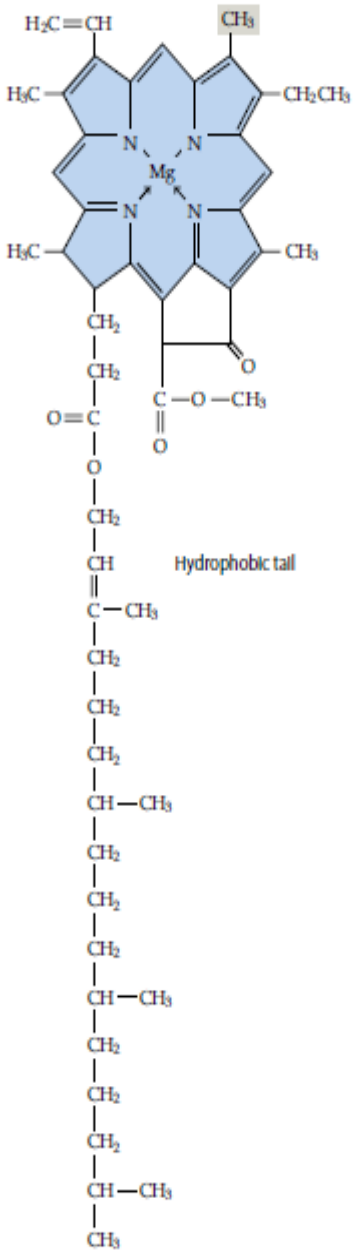


1. **Chlorophyll (Main photosynthetic pigment)**
2. **Carotenoids (Accessory pigment)**
3. **Phycobilins (Accessory pigment)**

1. Chlorophyll

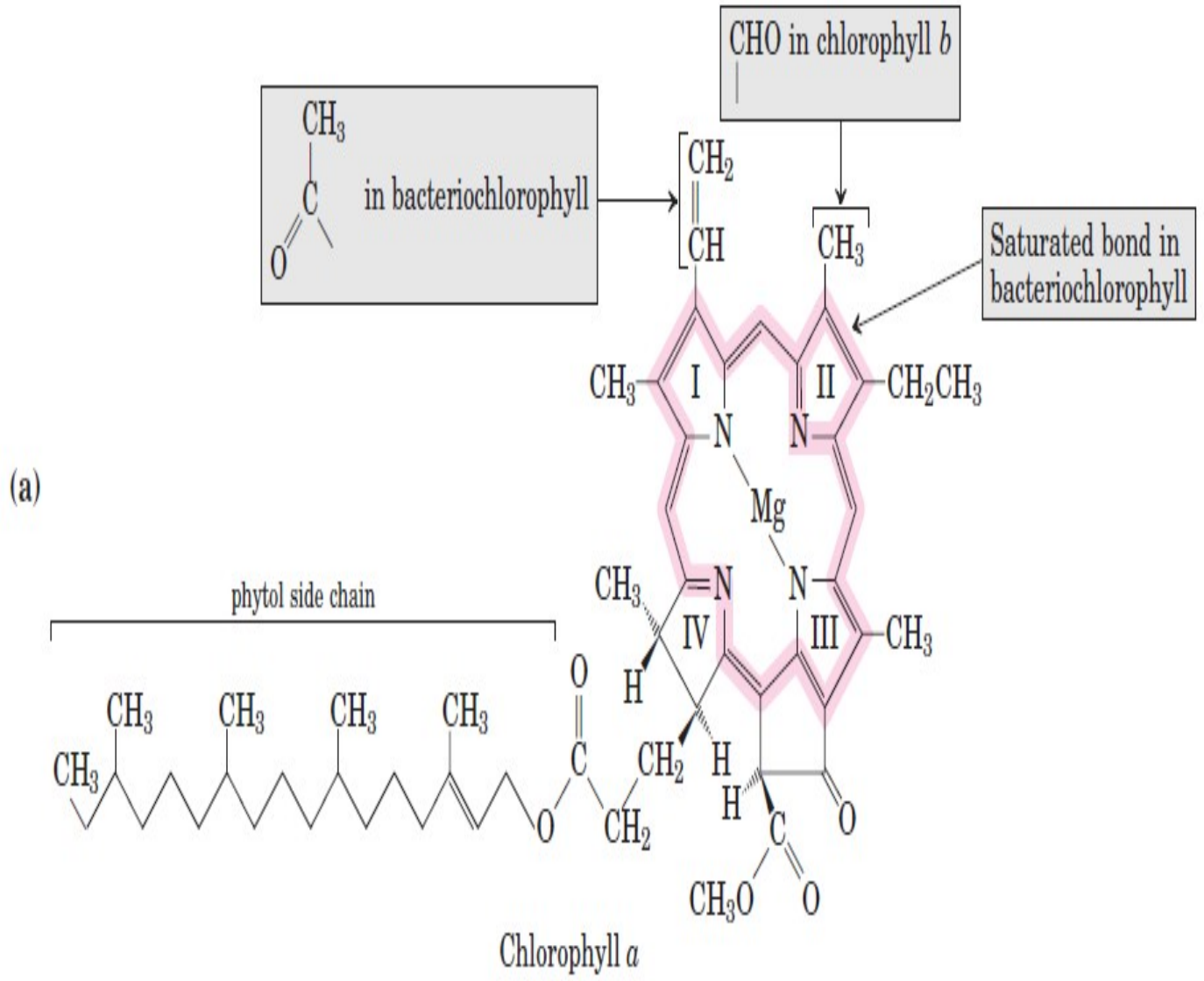
- Present in all oxygenic photosynthetic organisms
- **Types:** Chl A, Chl B, Chl C, Chl D
- Lipid soluble pigment
- Absorbs light most strongly in blue and red portion and poorly in green portion of electromagnetic spectrum

- **Chlorophyll is a Chlorin/ Porphyrin derivative:**
  - **Chlorin:**
    - Large heterocyclic aromatic ring
    - 3 pyrrole ring + 1 pyrroline ring linked with 4 Methine linkages
    - Chl A, Chl B, Chl D are chlorin derivative
  - **Porphyrin:**
    - 4 Pyrrole rings
    - 4 pyrrole/ pyrrole like ring= tetrapyrrole compounds
    - Chl C is a porphyrin derivative

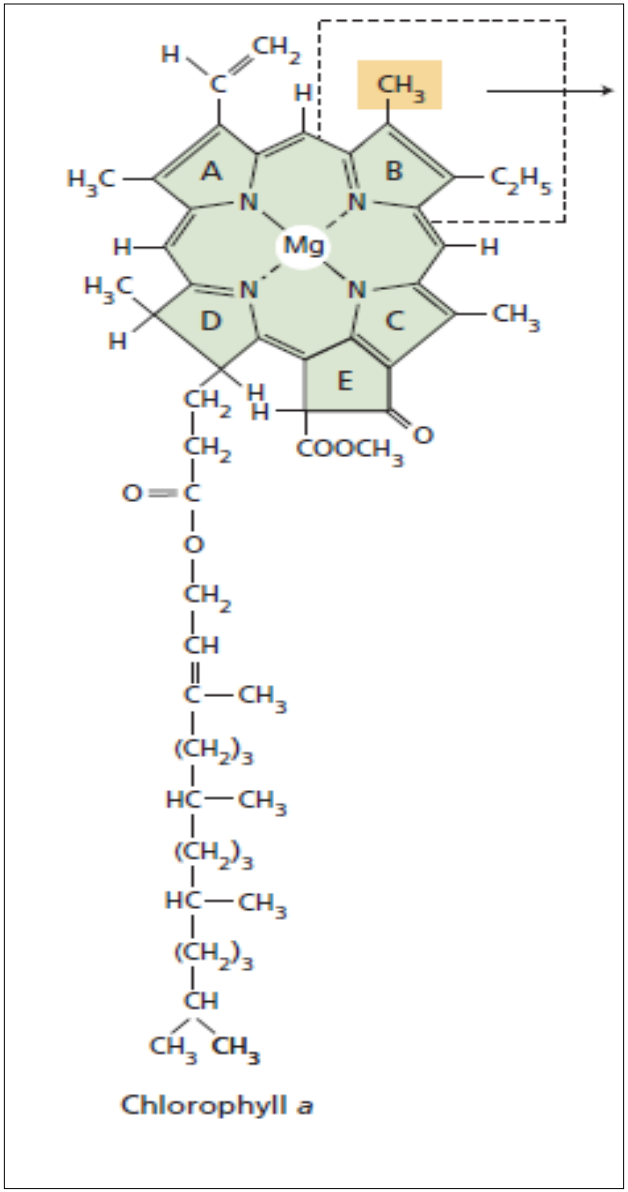


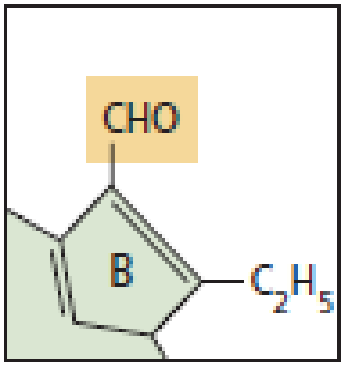
Structure of Chlorophyll

- Large heterocyclic aromatic ring
- 3 **Pyrrole Ring (5 atom ring with 4 carbon atoms+ 1 nitrogen atom)** + 1 **Pyrroline Ring**
- These rings are linked with 4 Methine (=CH-) linkages
- Central metal ion **Mg^{2+}** (covalently bound with 2 N-atoms; coordinately bound to other 2N-atoms of tetrapyrrole ring
- **Cyclopentanone ring (Ring V)**- Attached to pyrrole ring III
- **Phytol side**

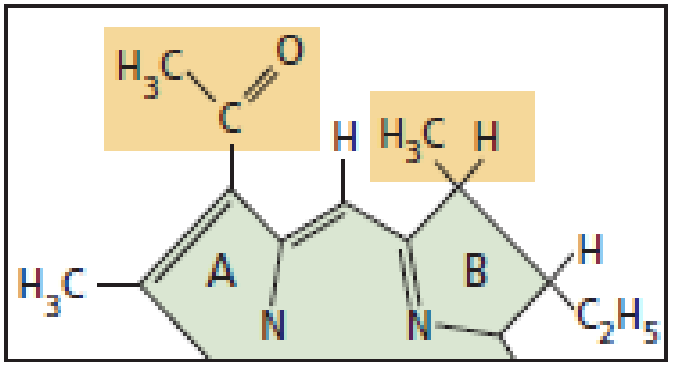


Chlorophyll *a*





Chlorophyll *b*



Bacteriochlorophyll *a*

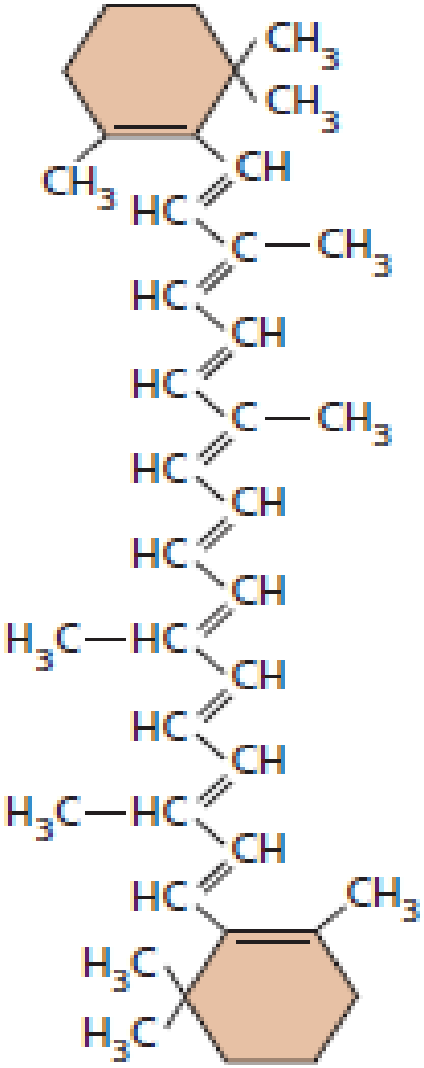
Chl A	***Chl B***
$C_{55}H_{72}O_5N_4Mg$	$C_{55}H_{70}O_6N_4Mg$
In pure state, Chl A – Green Blue	In pure state, Chl B- Olive Green
Essential photosynthetic pigment	Accessory photosynthetic pigment
Pyrrole ring II- (-$\mathbf{CH_3}$)	Pyrrole ring II- (-**CHO**)
Absorbs more Red wavelength	Absorbs more violet blue wavelength

2. Carotenoids

- Accessory pigment (**light harvesting pigment**)
- Long chain, conjugated hydrocarbons
- String of isoprene residues
- C_{40} terpenoid compounds
- Lipid soluble pigment
- **Absorption:** 400-500nm

- **Subdivided into 2 classes:**
  - Xanthophylls
  - **Carotenes**

- **Functions:**
  - Capture light energy and feeds it to the photochemical reaction center
  - Provide protection against photo-oxidation damage (**Photoprotection**)
  - Acts as **Quencher** as well as **Scavenger**



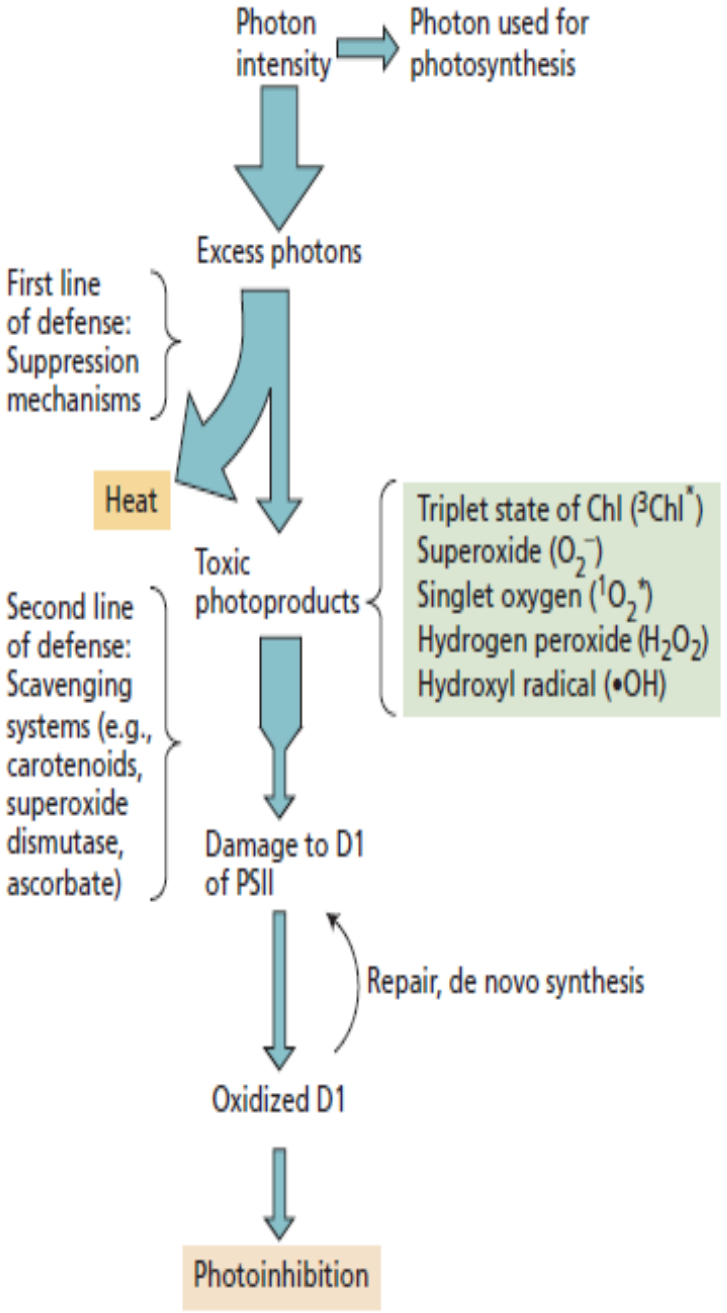
β-Carotene

Photoprotection mechanism

Energy stored in chlorophyll in excited state → **Rapidly dissipated by excitation transfer/ photochemical reaction**

PHOTOCHEMICAL QUENCHING

- Carotenoids exert **Photoprotection action** and prevent damage by **removing excess of energy**
- Rapidly quenching excited state of chlorophyll
- The excited state of carotenoids does not have sufficient energy to form singlet oxygen
- So decays back to ground state while loosing its energy as heat
- The process is called **Non Photochemical quenching**
- 3 xanthophylls involved in non-photochemical quenching are:
  1. **Violaxanthin**
  2. **Antheraxanthin**
  3. **Zeaxanthin**



Protection against photo damage is multilevel process

1st line of defense: Suppression of damage by quenching of excess excitation as heat

If this defense is not sufficient and toxic photoproducts form

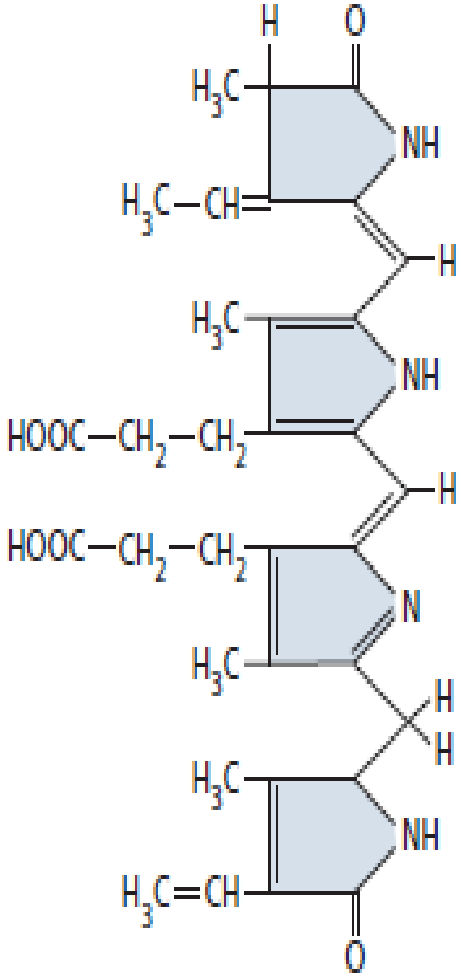
2nd Line of defense: A variety of scavenging systems eliminate reactive photoproducts

If the 2nd line of defense fails= photoproducts damage the D1 protein of PSII

The damage leads to **Photoinhibition**

3. Phycobilins

- Non-cyclic
- Linear tetrapyrrole structures
- Structurally similar to bile pigment bilirubin
- 3 photosynthetic phycobilins are:
  - **Phycoerythrobilin**
  - **Phycocyanobilin**
  - **Allophycocyanobilin**
- Phycobilins are present most abundantly in **Red Algae** and **Cyanobacteria**
- Unique because it has covalently bound (**thioether bond**) to certain water soluble proteins = **Phycobiliproteins**
- **Functions:**
  - Accessory photosynthetic pigment (Light Harvesting pigment) captures light energy, feed to chlorophyll molecules present at photochemical



Phycoerythrobilin

Phycobiliproteins
Water soluble protein, aggregate into cluster which is found adhered to membrane (**phycobilisomes**)

Phycoerythrins
Protein + Phycoerythrobilin

Phycocyanins
Protein + Phycocyanobilin

Category	Chlorophyll				Carotenoids	Phycobilins
	A	B	C	D		
Plants						
Angiosperms	+	+	-	-	+	-
Gymnosperms	+	+	-	-	+	-
Pteridophytes	+	+	-	-	+	-
Bryophytes	+	+	-	-	+	-
Algae						
Green algae	+	+	-	-	+	-
Red algae	+	-	-	+	+	+
Brown algae	+	-	+	-	+	-
Protists						
Diatoms	+	-	+	-	+	-
Dinoflagellates	+	-	+	-	+	-
Euglena	+	+	-	-	+	-
Cyanobacteria	+	-	-	-	+	+

(+) PRESENT (-) ABSENT

Location of photosynthetic pigments

Organisms	***Location***
Oxygenic photosynthetic organisms	Thylakoid membrane
Anoxygenic photosynthetic organisms	
1. **Green sulfur bacteria**	Plasma membrane and vesicles called **Chlorosomes** attached to plasma membrane
2. **Purple sulfur bacteria**	Invaginations of plasma membrane called **Chromatophores**
Eukaryotic photosynthetic organisms	Thylakoid inside chloroplast
Cyanobacteria (except ***Gleobacter violaceus***)	Internal system of thylakoid membrane in cytosol

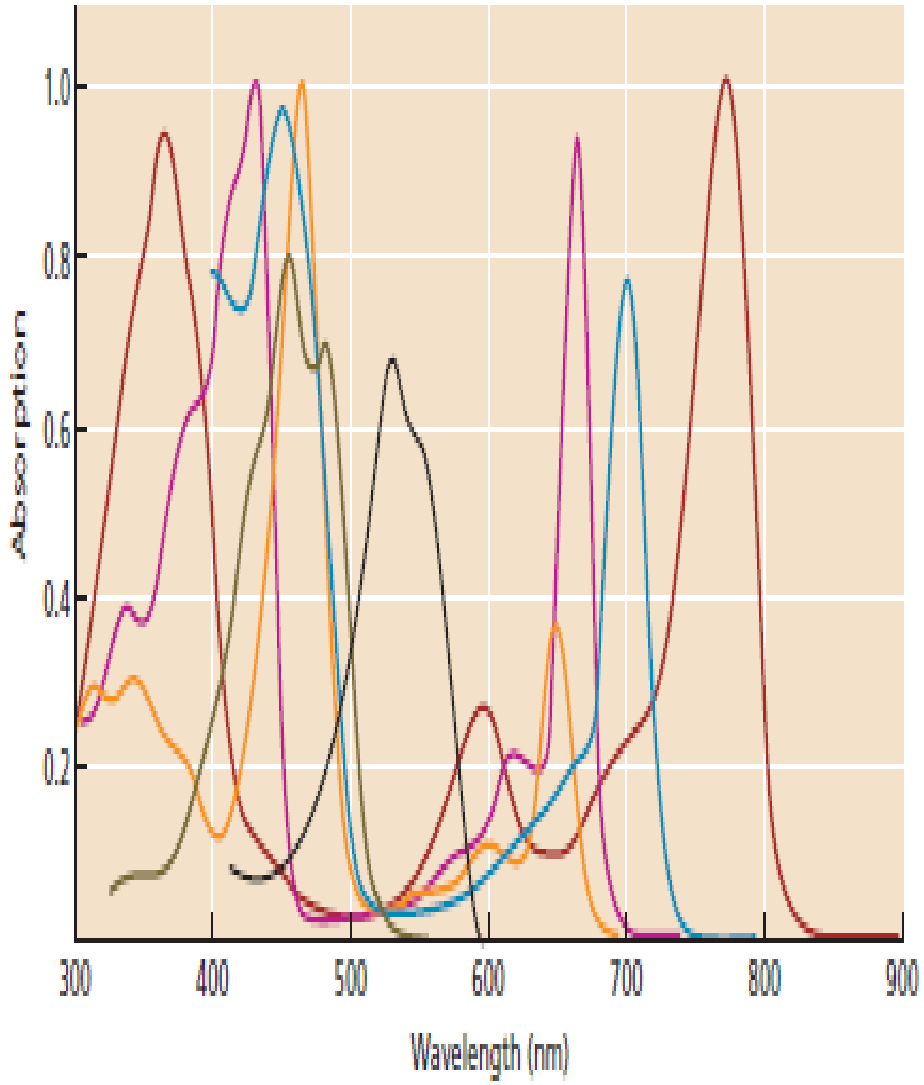
Absorption spectra and Action spectra

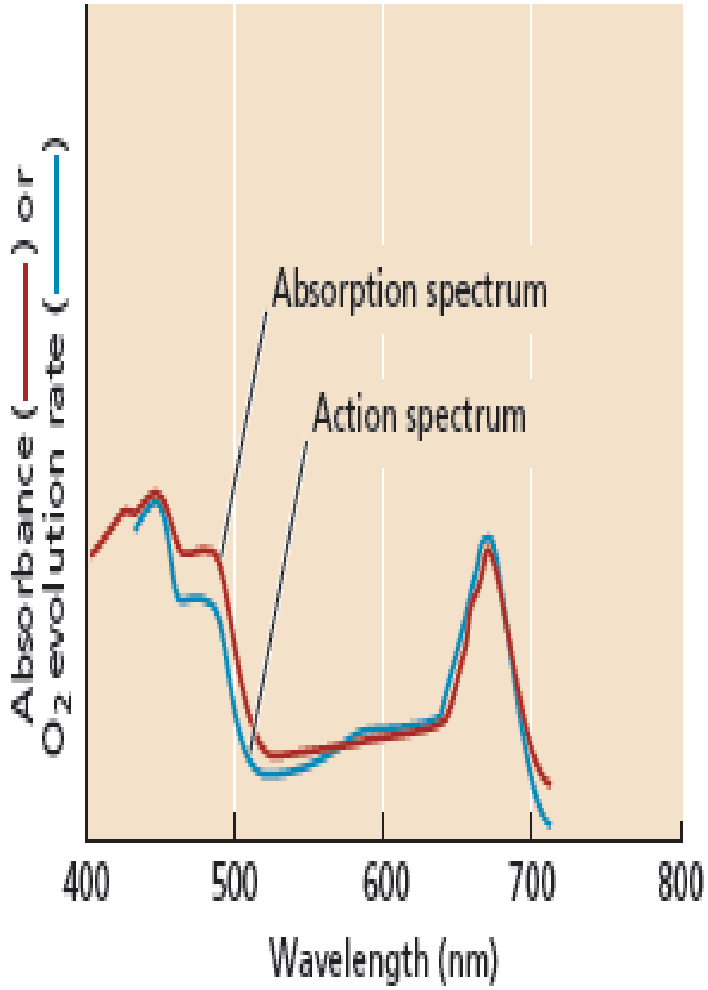
Photosynthetic pigment → Absorbs → **Electromagnetic radiation in visible and Infrared region**

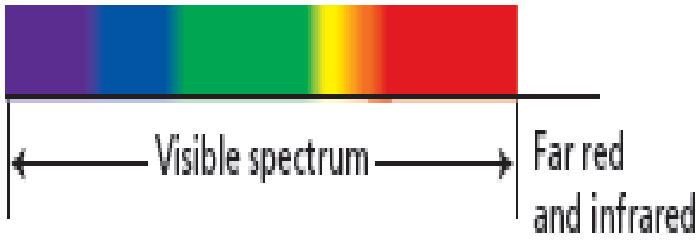
Graph

- Showing degree of **absorption of light by pigment** as a function of wavelength
  Absorption spectrum
- Showing degree to which **different wavelength affect photochemical reaction**
  (Response to light as a function of wavelength)
  Action spectrum

Action spectra = Absorption spectra
If pigment used to obtain absorption spectrum are same as those that causes Oxygen evolution

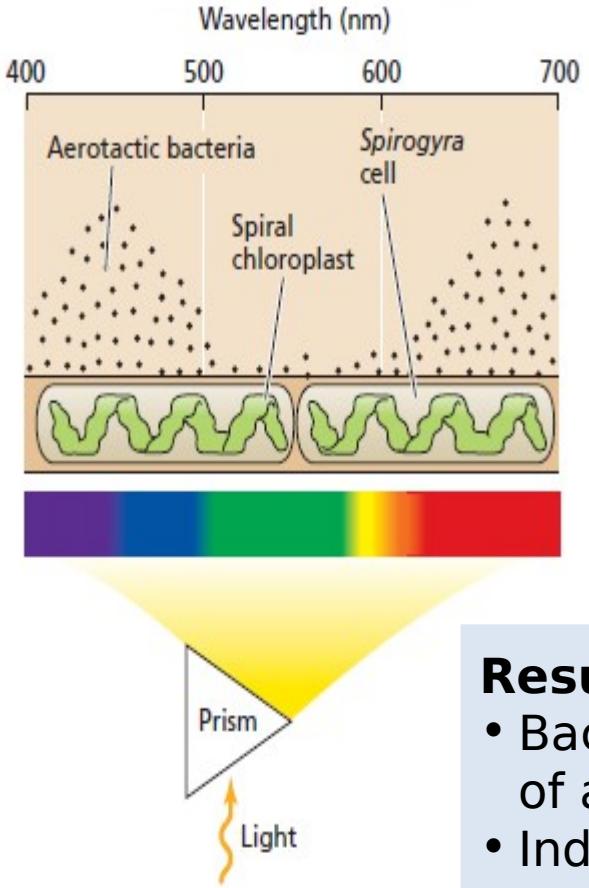

Absorption
1.0
0.8
0.6
0.4
0.2
300
400
500
600
700
800
900
Wavelength (nm)
β-Carotene
Chl a
Chl b
Bchl a
Chl d
Phycoerythrobilin


Absorbance (——) or
O2 evolution rate (——)
Absorption spectrum
Action spectrum
400
500
600
700
800
Wavelength (nm)


Visible spectrum
Far red
and infrared

Engelmann's experiment

- Action spectra relate light absorption to photosynthetic activity
- Determined 1st action spectra by measuring oxygen evolution at different wavelength of light



White light

Pris m

Filamentous green algae= *Spirogyra*

Aerobic oxygen seeking bacteria = concentrates near an Oxygen source

Result:

- Bacteria congregated in great numbers around parts of alga illuminated with blue or red light
- Indicates the effectiveness of light absorbed by accessory pigments in driving photosynthesis

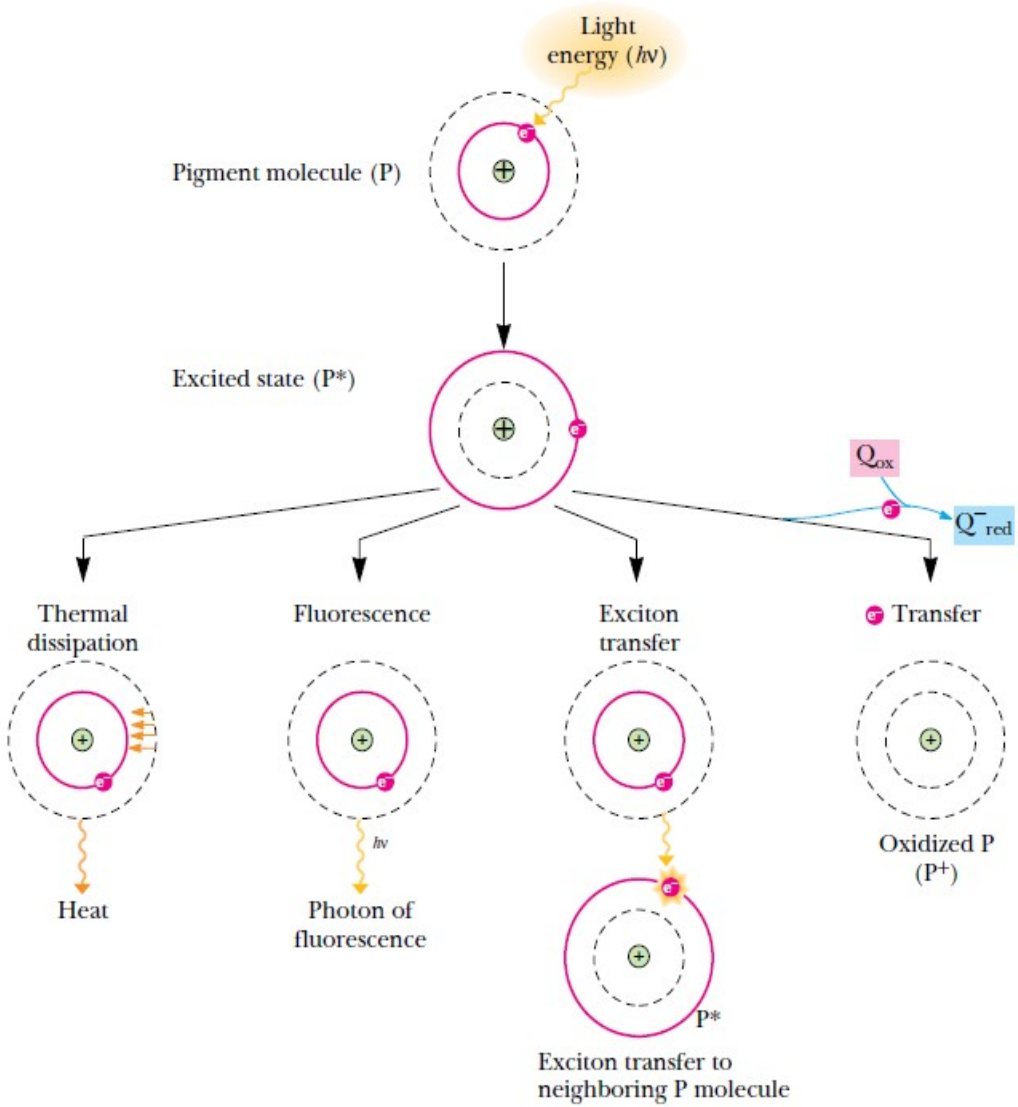
Fate of light energy absorbed by photosynthetic pigments

Photon = Quantum of light energy

Absorption Of Light → Chlorophyll → Excited chlorophyll to higher energy state (**unstable**)

Excited chlorophyll to higher energy state (**unstable**)
Has 3 fates

1. Converting extra energy into heat or combination of heat and light of longer wavelength= **Fluorescence**
2. Transferring energy directly to neighboring chlorophyll molecule
3. Transferring high energy electron to another nearby molecule (an electron acceptor) and then returning to original state by taking up low energy electron from some other molecule (electron donor)



Concept of photosynthetic unit

- **Robert Emerson**
- **William Arnold**

Cooperation of many chlorophyll molecule in energy conversion during photosynthesis

Suspension of alga ***Chlorella*** + Lights of saturating intensity (short; ~10 µsec) = Minimum amount of light needed to produce maximal oxygen production

1 Oxygen released → Absorption of 8 photons → 2400 Chlorophyll

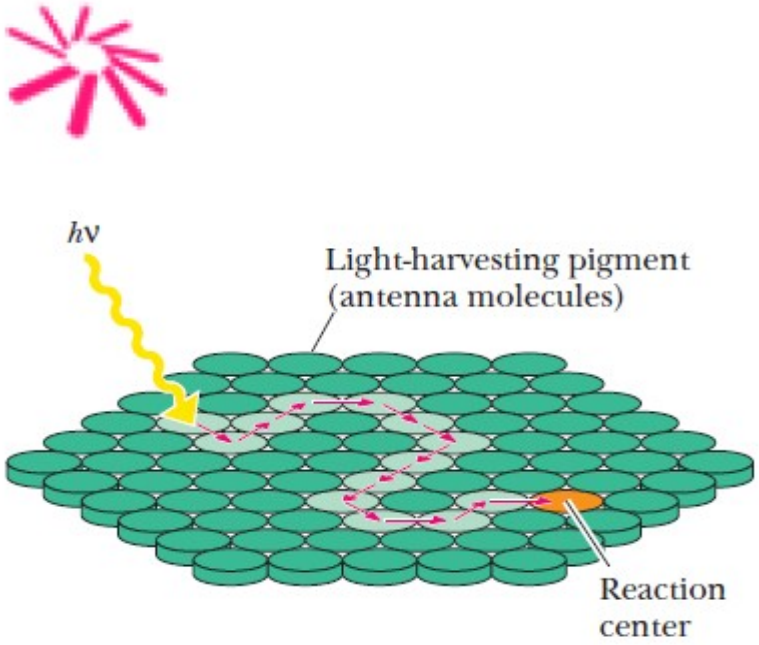


FIGURE 22.9 • Schematic diagram of a photosynthetic unit. The light-harvesting pigments, or antenna molecules *(green)*, absorb and transfer light energy to the specialized chlorophyll dimer that constitutes the reaction center *(orange)*.

Photosynthetic Unit

- Chlorophyll molecules
- Absorbs light and transfer excitation energy to **Reaction center** – special pair of Chl A molecules performs photochemical reactions

Hill reaction

- Light driven transfer of electrons from water to hill reagents (**non-physiological oxidants**)
- Oxygen produced from oxidation of water during photosynthesis occurs separately from process that reduces(or fixes) carbon dioxide into sugars

$$2H_2O + 2NADP^+ \xrightarrow{\text{Light}} 2NADPH + 2H^+ + O_2$$

Oxygenic photosynthesis

- Light energy-captured
- Converted into ATP
- Production of Oxygen
- Due to photo oxidation/ photolysis of water

Anoxygenic photosynthesis

- Light energy –captured
- Converted into ATP
- No production of Oxygen
- Ex. ***Chromatium vinosum*** uses H_2S (Electron donor) forms sulfur

Concept of pigment system

Robert Emerson and Charlton Lewis

Experiment-1

- Examination of Action spectrum in visible region- for oxygen evolution in number of oxygen molecules produced per photon absorbed in ***Chlorella pyrenoidosa***
- **Results found:**
  - Quantum yield constant upto 680nm
  - Beyond 680nm= Quantum yield declined sharply = Drop in **Far Red Region** of spectrum= **Red Drop Phenomenon**
  - Light above 680nm = less efficient
  - **Quantum yield** = Number of oxygen molecules produced per photon absorbed
  - **Quantum requirement** = It is

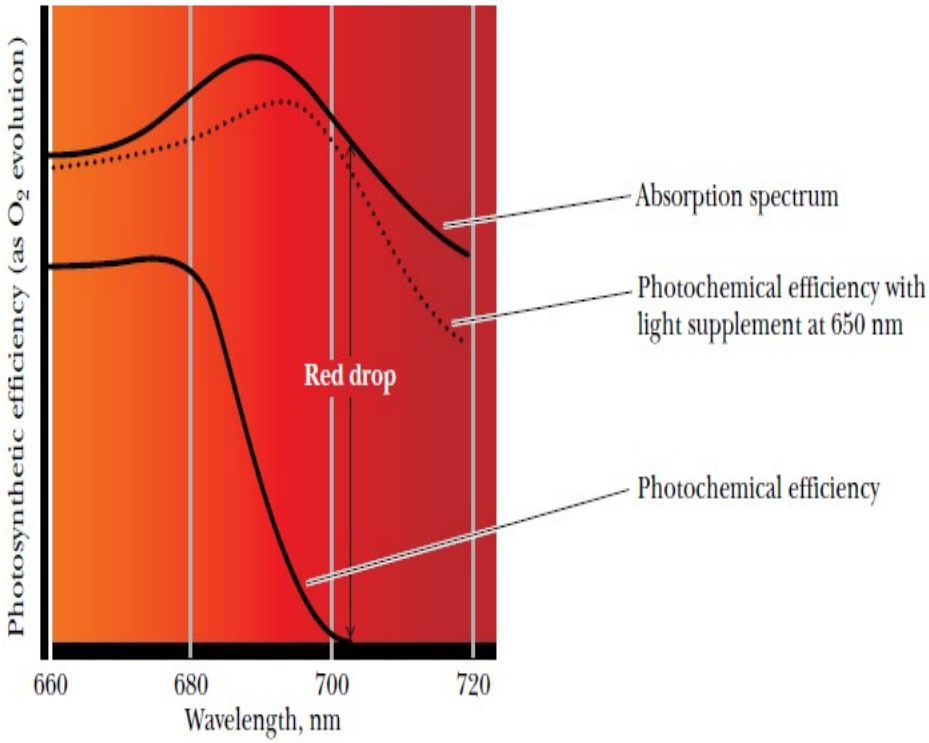
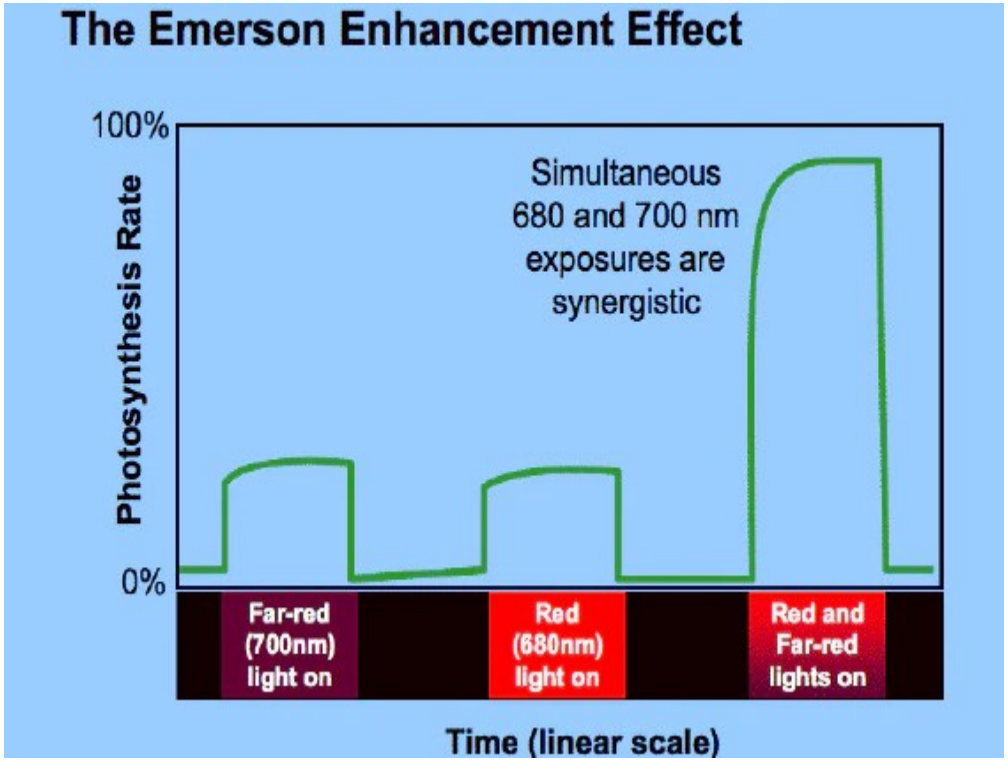
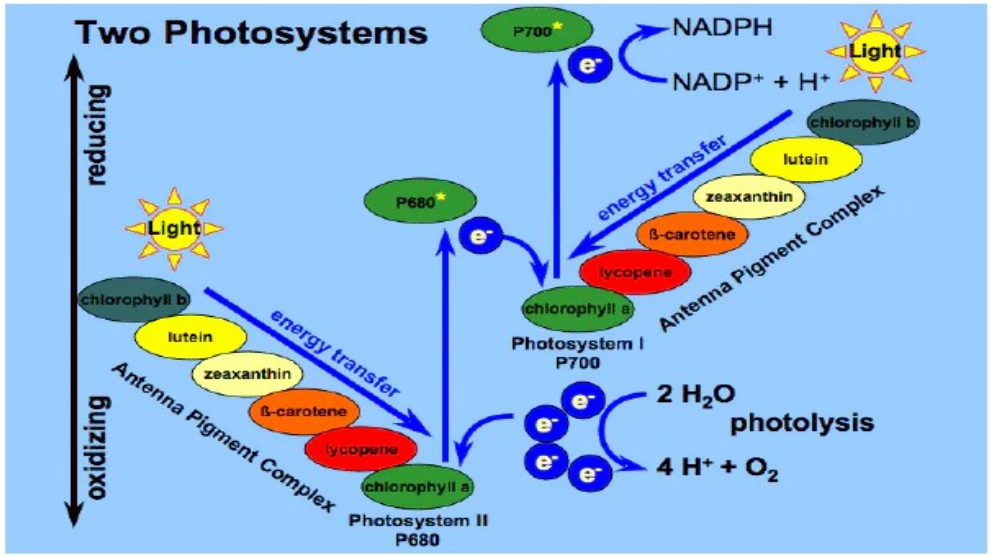


FIGURE 22.10 • The photochemical action spectrum of photosynthesis. The quantum yield of photosynthesis as a function of wavelength of incident light shows an abrupt decrease above 680 nm, the so-called *red drop*.

Experiment-2

- 2 beams of light (**Red region (<680nm)** and **Far red region (>680nm)**
- 2 beams applied simultaneously = Rate of photosynthesis = 2-3 times greater than sum of rates obtained separately
- **Emerson enhancement effect**
- **Results found:**
- Photosynthesis involves 2 Photosystems
- 1- driven by light of long wavelength **(>680nm)**
- 2- driven by light of short wavelength **(=/< 680nm)**
- **Pigment Systems = Pigment Molecule + Protein Pigment**





https://www.linkedin.com/pulse/how-grow-better-leveraging-the-emerson-enhancement-effect-with-led-grow-lights?srsltid=AfmBOoqDKsi0s5KaAipRkhgaDnJG4NgM3mYbw9FuyiHcEakr-q0s7vY7

Pigment system = Pigment molecule + proteins (pigment-protein complex) or Photosystems

Components of pigment system :

1. **Photochemical reaction center**
   - Protein pigment complex
   - Carries out photochemical reaction
   - It has 2 centers-
     - **Fe-S** (Type I)
     - **Pheophytin-Quinone** (Type II)
2. **Antenna complex**
   - Light harvesting complex
   - Consists of pigment protein complexes
   - Has 2 components-
     - **Core/Inner antenna**
     - **Peripheral/ Outer antenna**

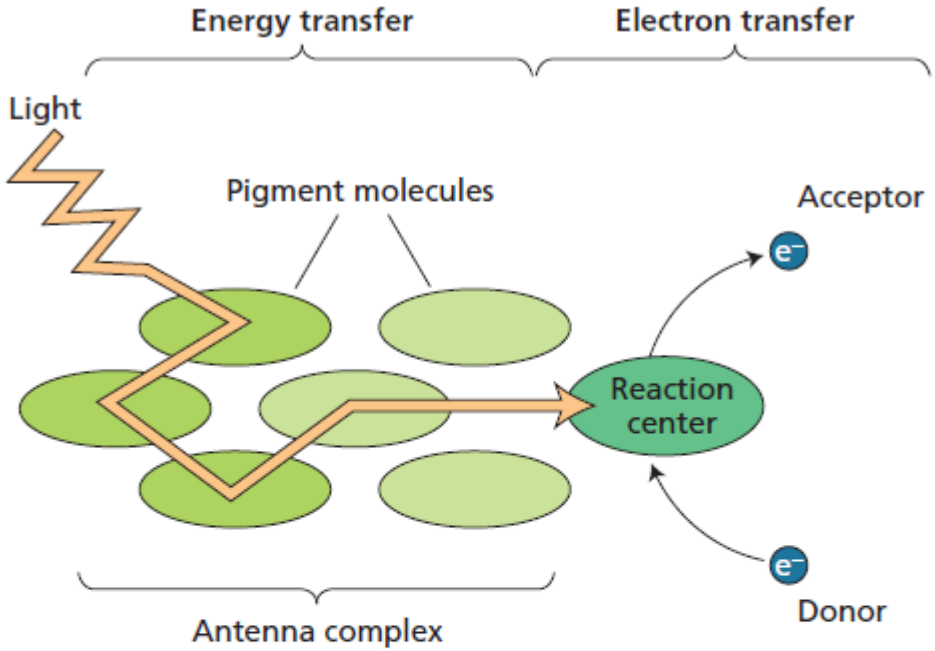


Figure 7.10 Basic concept of energy transfer during photosynthesis. Many pigments together serve as an antenna, collecting light and transferring its energy to the reaction center, where chemical reactions store some of the energy by transferring electrons from a chlorophyll pigment to an electron acceptor molecule. An electron donor then reduces the chlorophyll again. The transfer of energy in the antenna is a purely physical phenomenon and involves no chemical changes.

Resonance energy transfer

Captures light energy and feeds to reaction cent

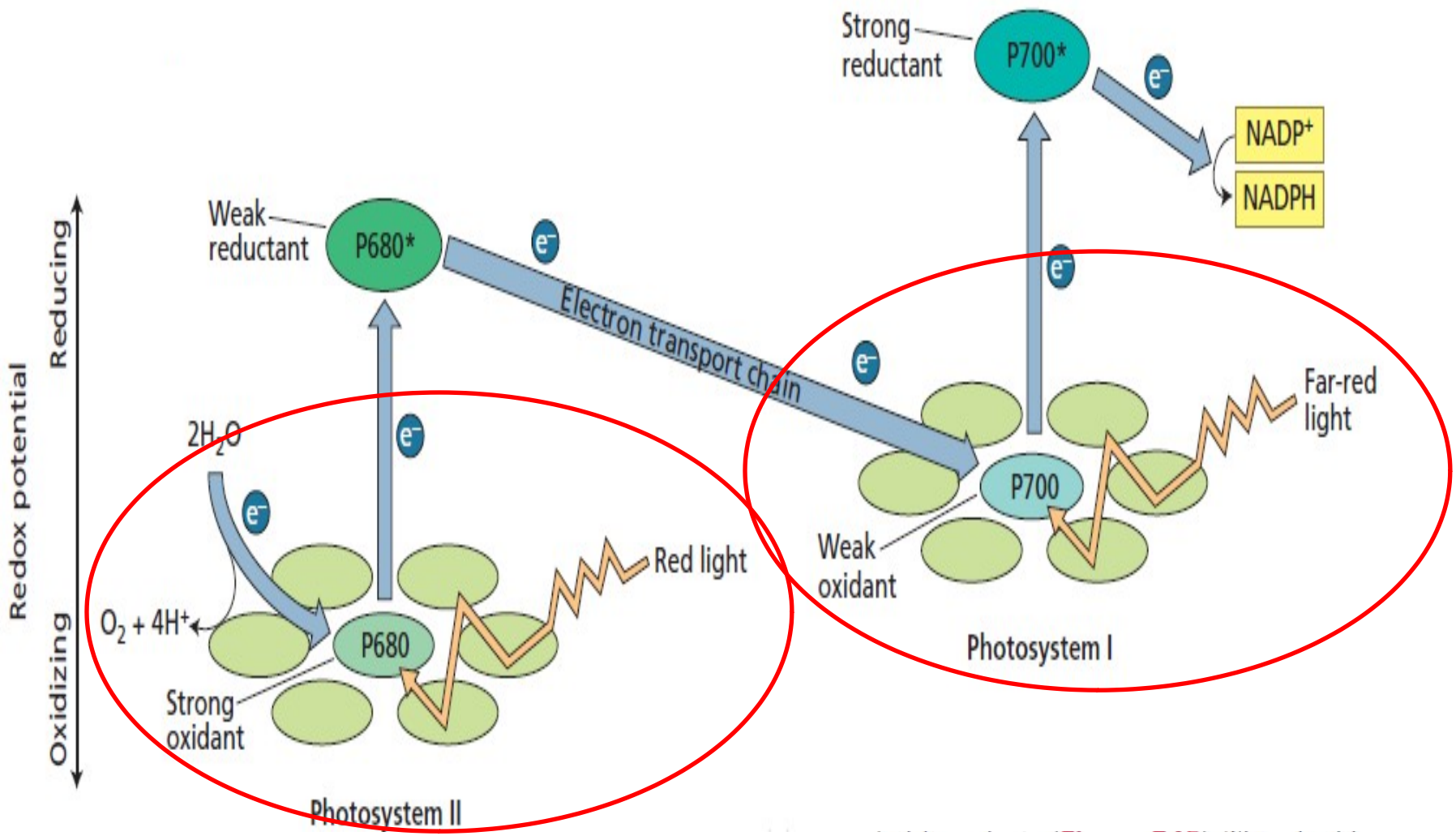
In oxygenic photosynthetic organisms (plants and cyanobacteria)

- 2 photo systems
  - **PS I- Fe-S reaction center**
  - **PSII- Pheophytin-**

hotosystems are found in plants and other photosynthetic cyanobacteria

SII

SI



PS II

- **Pheophytin Quinone center (P-680)**
- Effectively absorbs light of wavelength **680nm**
- Associated with light harvesting antenna complexes:
  - Core antenna
  - Peripheral antenna= **LHC II**
- Chl A + Pheophytin + Plastoquinone + cofactors
- In higher plants, the reaction center contains membrane proteins D1 and D2 , cytochrome b_{559} and other proteins along with Chl a molecules
- Chl a contains core antenna CP47 and CP43
- Peripheral antenna-Chl a/b binding proteins called **LHCII**

PS I

- **Fe-S reaction center (P-700)**
- Driven by light of wavelength **700nm**
- Associated with different proteins in thylakoid membrane
- Core antenna- integral part
- Peripheral antenna= **LHC I**
- Chl A + Carotenoids+ Phylloquinone + Fe-S center+ Cofactors

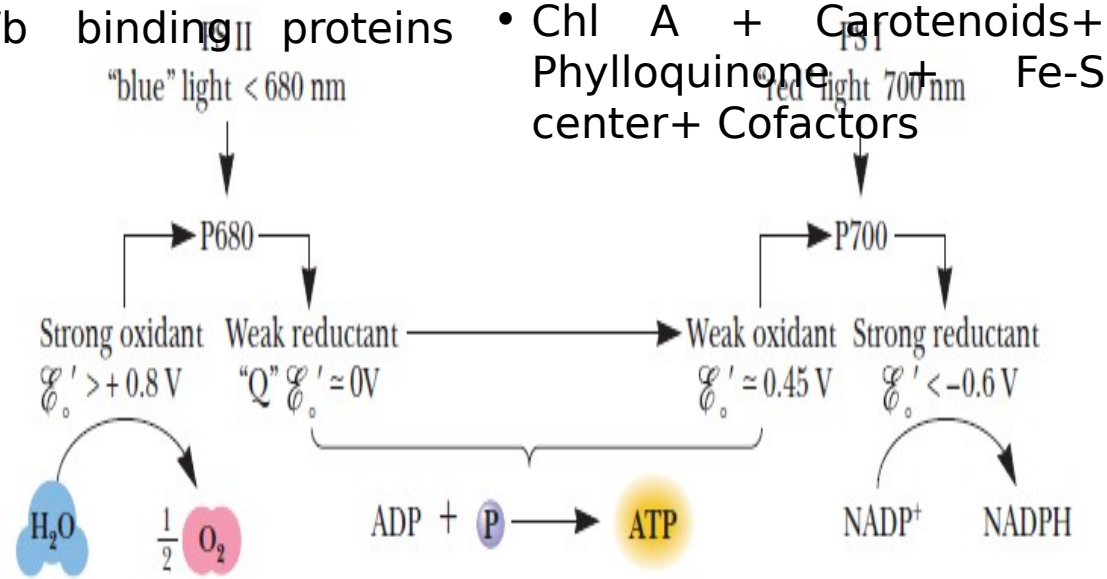


FIGURE 22.11 • Roles of the two photosystems, PSI and PSII.

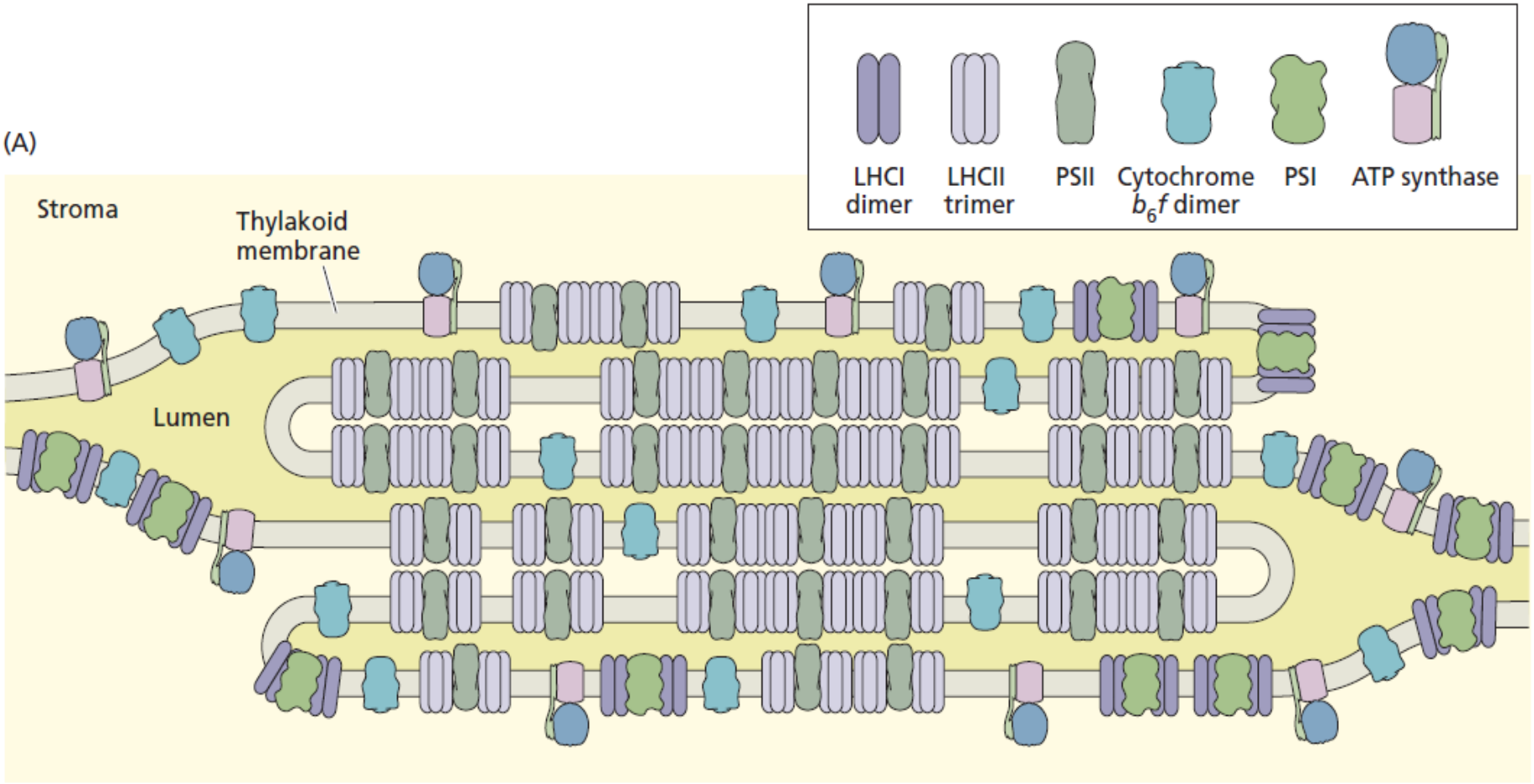
tribution of Photosystems (PSI and PS II)

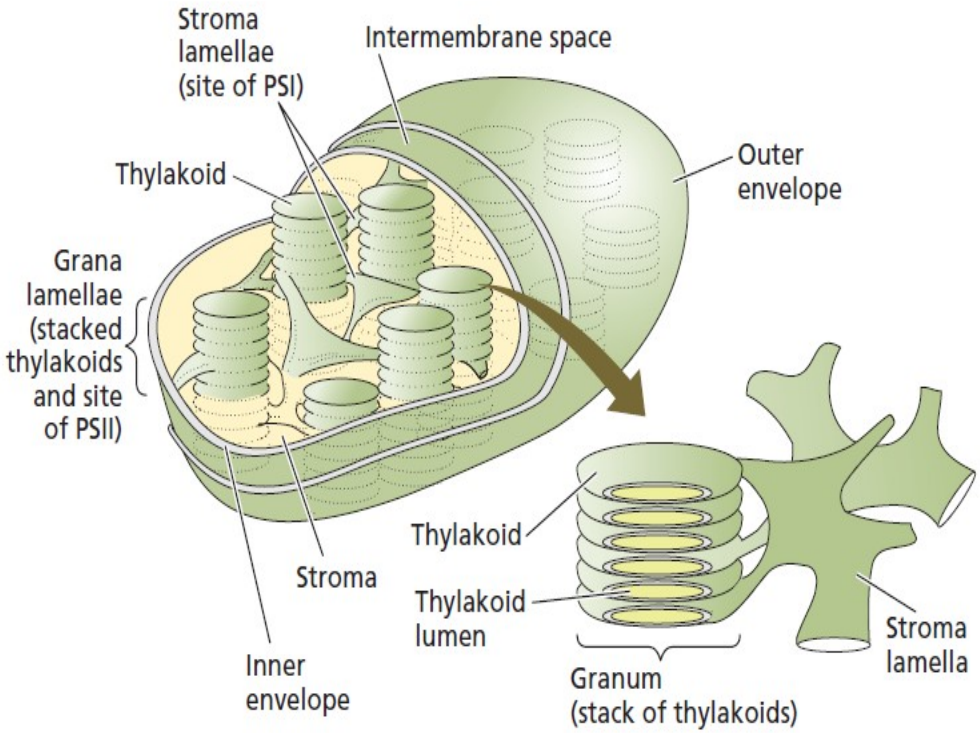
PS II

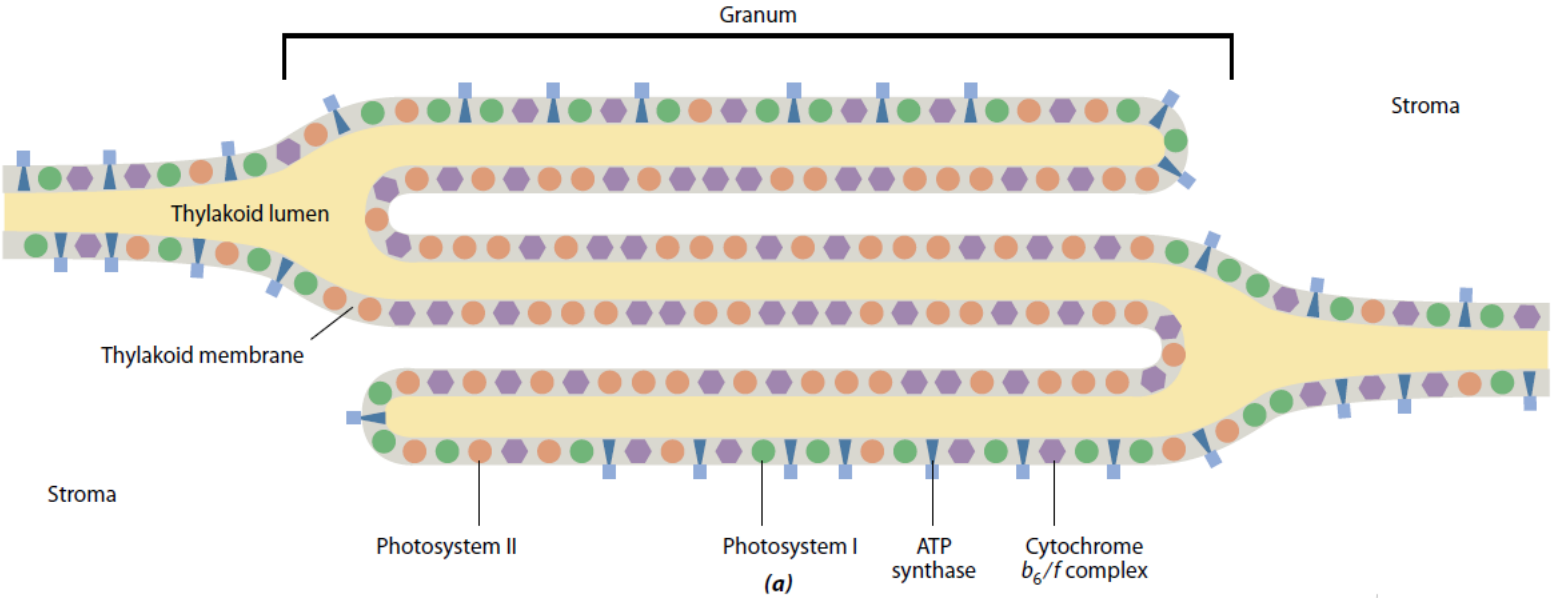
- Appressed membranes of Grana thylakoid

PS I

- Non- Appressed membranes of Grana thylakoid (Grana lamellae)
- Stroma thylakoid (stroma lamellae)




Stroma lamellae (site of PSI)
Intermembrane space
Thylakoid
Outer envelope
Grana lamellae (stacked thylakoids and site of PSII)
Thylakoid
Stroma
Thylakoid lumen
Inner envelope
Granum (stack of thylakoids)
Stroma lamella


Granum
Stroma
Thylakoid lumen
Thylakoid membrane
Stroma
Photosystem II
Photosystem I
ATP synthase
Cytochrome b_6/f complex
(a)

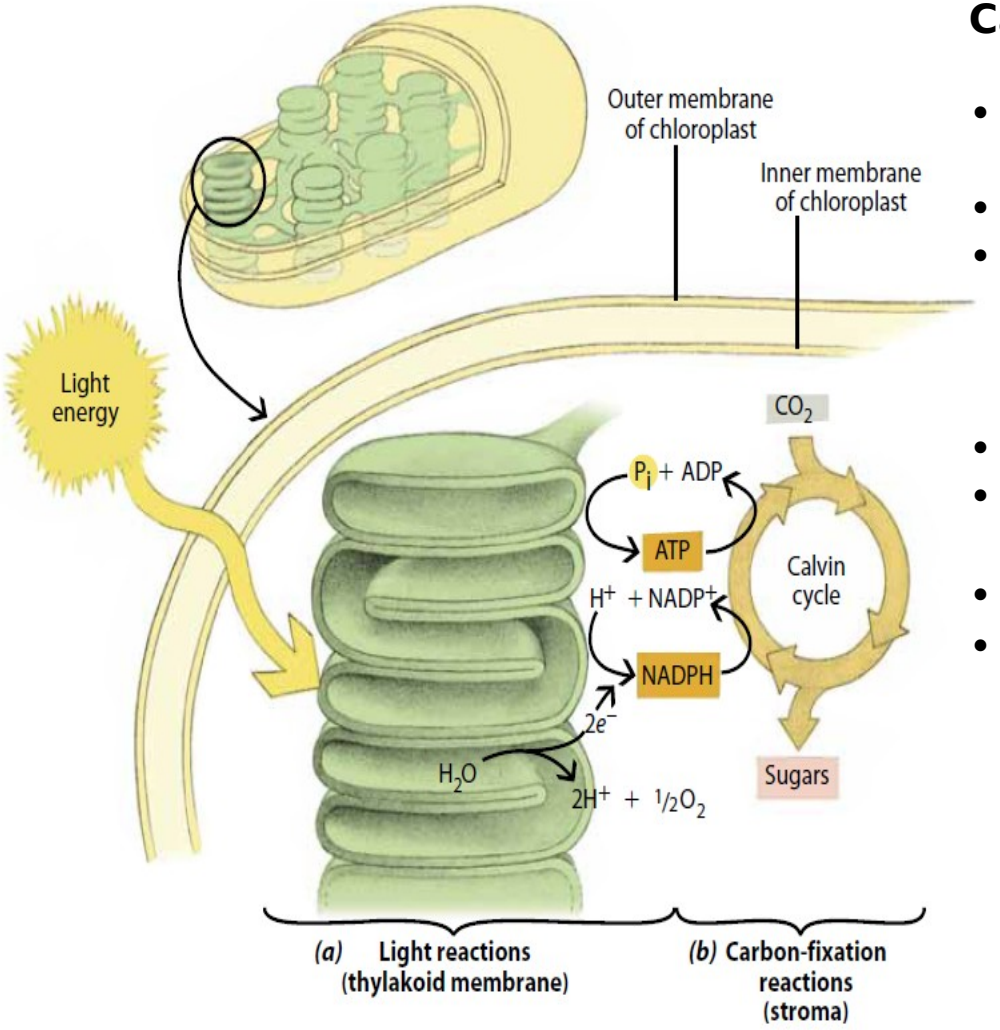
Rashmi MC

Photosynthesis in green plants

- 2 stages
  1. **Light reaction (dependent on light)**
  2. **Carbon-fixation reactions**

Light reactions

- Light **dependent** (Photochemical reactions)
- **Thylakoid**
- Makes **NADPH** and **ATP**
- **Thylakoid reaction**
- Oxidation of water, reduction of **$NADP^+$ and ATP formation**
- **ADP→ATP** Photophosphorylation
- Utilize **light energy**



Carbon-fixation reaction

- Light **independent** reactions
- **Stroma**
- Makes **glyceraldehyde-3-phospohate** (triose phosphate)
- **Stroma reaction**
- Carbon reduction and carbon fixation
- **CO_2→ G_3P**
- Utilize **ATP** and **NADPH**

Light reactions:

- Electron flow starts
- 2 types of electron flow
  1. **Noncyclic**
  2. **Cyclic**

Non cyclic

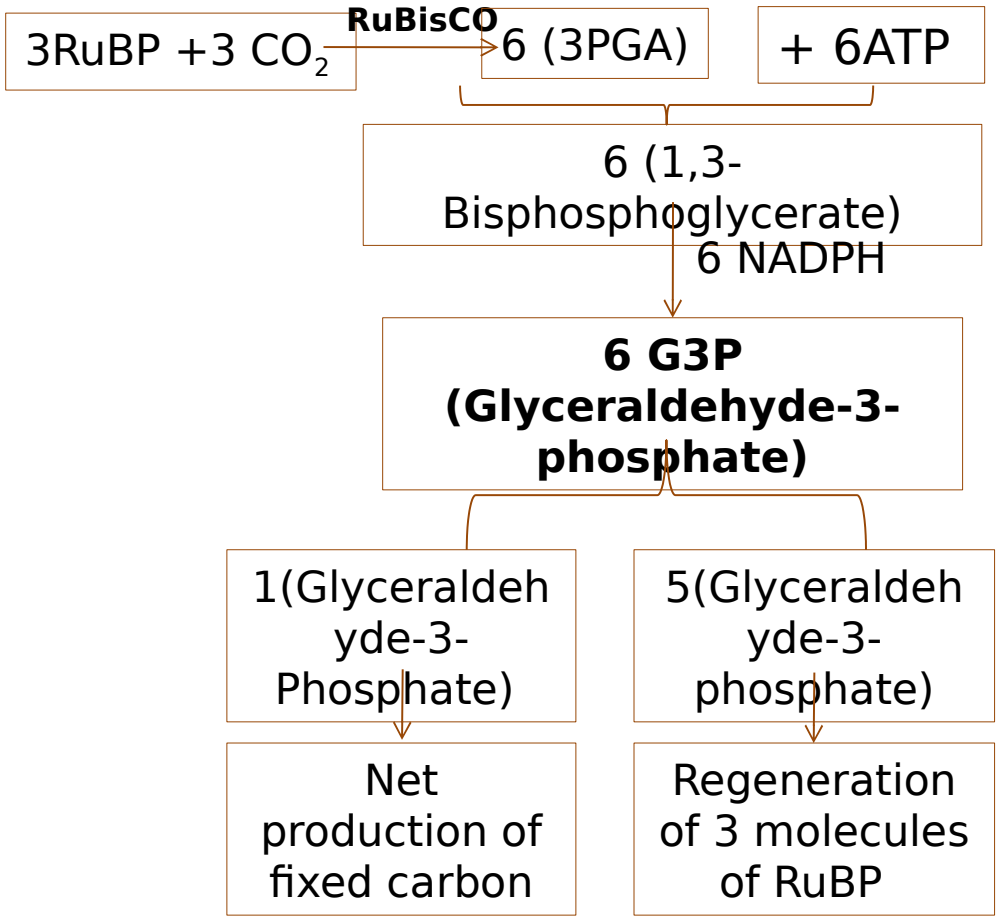
- Light induced electron transport
- **Water→ $NADP^+$**
- Photolysis of water, Evolution of oxygen , synthesis of NADPH
- 2 systems- PS II and PSI
- **H_2O→ PSII → PSI → $NADP^+$**
- Generation of proton motive force
- Synthesis of ATP due to light induced non cyclic electron flow = **Non cyclic photophosphorylation**

Cyclic

- Light induced electron transport
- **P700 of PSI → Cyt b_6f complex → back to P700**
- No photolysis, No oxygen evolution, no synthesis of NADPH
- Only one system- P700 (PSI)
- **P700→ P700* → A_0 → A_1 → Fe-S →**

Light independent reaction:

- Utilization of NADPH and ATP to fix the carbon in 3 phases
  1. **Carboxylation**
  2. **Reduction**
  3. **Regeneration of RuBP**



Light reaction

In oxygenic photosynthetic organisms water is electron donor
Splitting of 2 water molecules yields 4 electrons, 4 protons and molecular oxygen

$2\ H_2O \xrightarrow{4hy} 4H^+ + 4e^- + O_2$

Absorption of photon by **PSII**

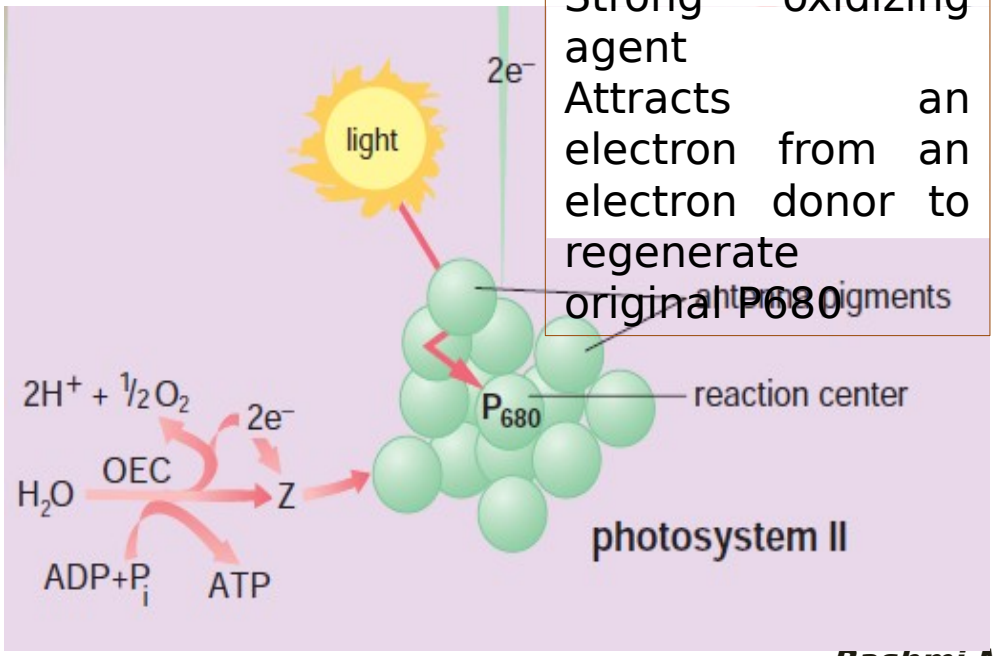
P680 Excited→ P680*

e^-

Pheophytin

P680* converted into $P680^+$
Strong oxidizing agent
Attracts an electron from an electron donor to regenerate original P680

- Splitting of water
- Catalyzed by protein complex (**Oxygen evolving complex**)
- Located in luminal surface of thylakoid membrane
- Contains Proteins (Manganese stabilizing protein PsbP and PsbQ) + 4 Manganese + Calcium ion + Chlorine ion + Bicarbonate ion



- 4 electrons abstracted from photolysis of 2 water molecules do not pass directly to $P680^+$
- Can accept only one electron at a time

Oxygen evolving complex (OEC)

- It cycles through a series of oxidation states – releases electron one at a time $P680^+$
- $4e^-$ one by one

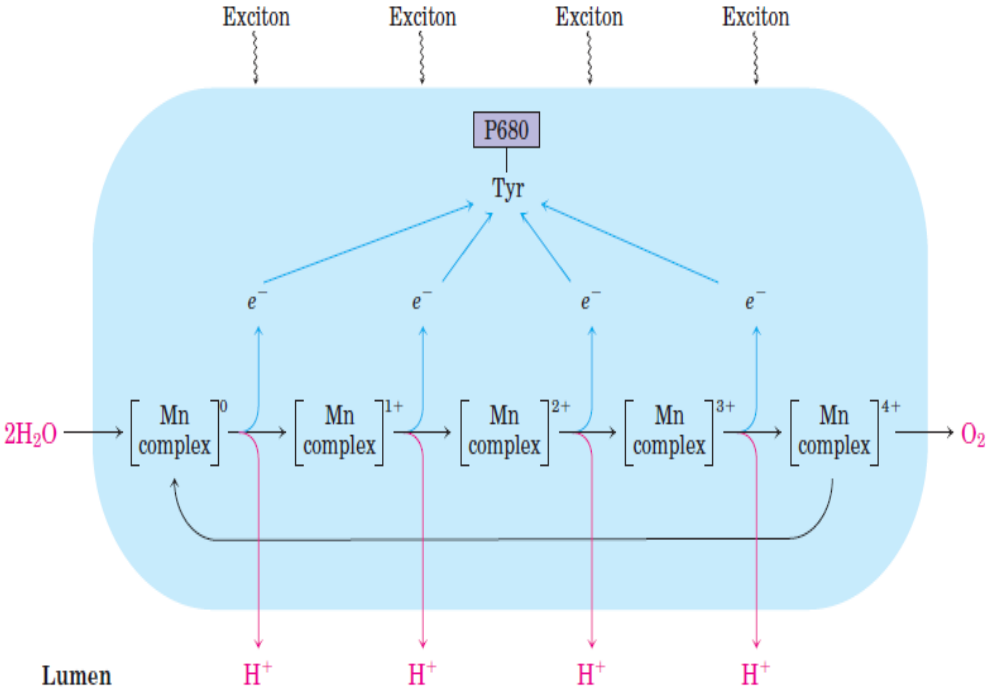
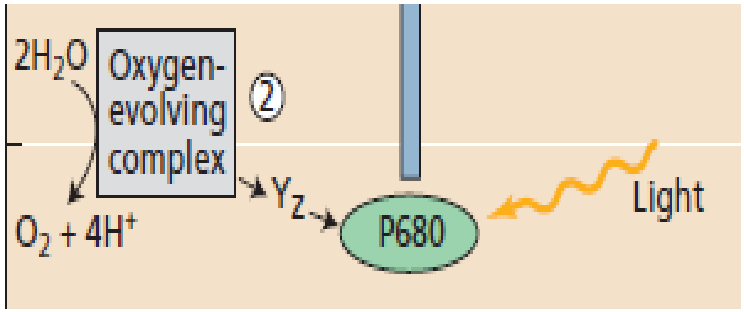


FIGURE 19-56 Water-splitting activity of the oxygen-evolving complex. Shown here is the process that produces a four-electron oxidizing agent—believed to be a multinuclear center with several Mn ions—in the water-splitting complex of PSII. The sequential absorption of four photons (excitons), each absorption causing the loss of one electron from the Mn center, produces an oxidizing agent that can remove four electrons from two molecules of water, producing O_2. The electrons lost from the Mn center pass one at a time to an oxidized Tyr residue in a PSII protein, then to $P680^+$.



- $P680^+$ Sequential absorption of 4 photons
- Each causing loss of 1 electron from Mn center
- Produces an oxidizing agent that can take 4 electrons from 2 molecules of H_2O
- Producing O_2
- Electrons lost from Mn center pass one at a time to **Tyr** residue (Y_2) in D1 protein

e⁻ → P680* → Pheophytin → Reduced **Pheophytin**

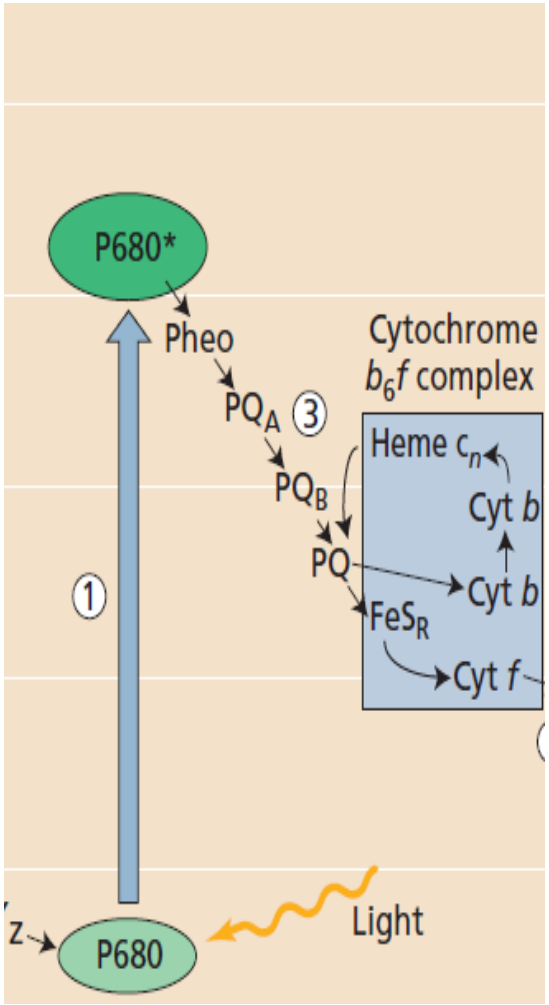
Plastoquinone
Present in PSII both as a tightly and loosely bound electron carriers

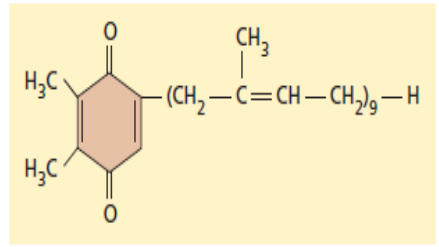
Q_A
Primary Quinone bound to D2 reduced to **Plastosemiquinone**

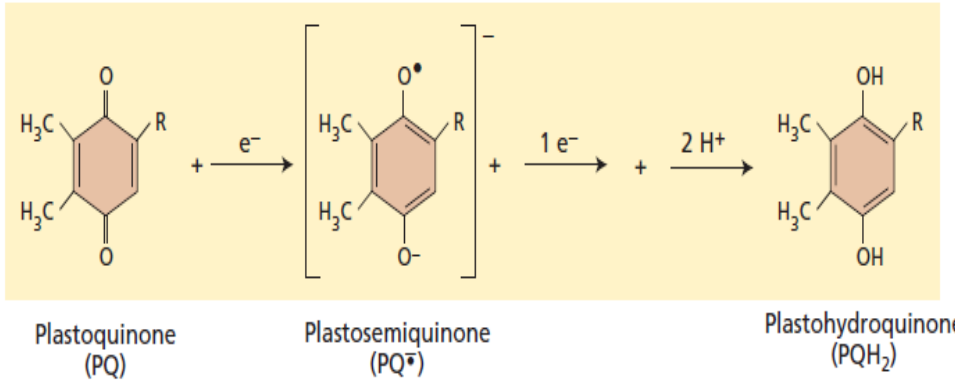
Q_B
Secondary Quinone bound to D1 accepts 2 electrons 2 protons fully reduced – **Plastoquinol**

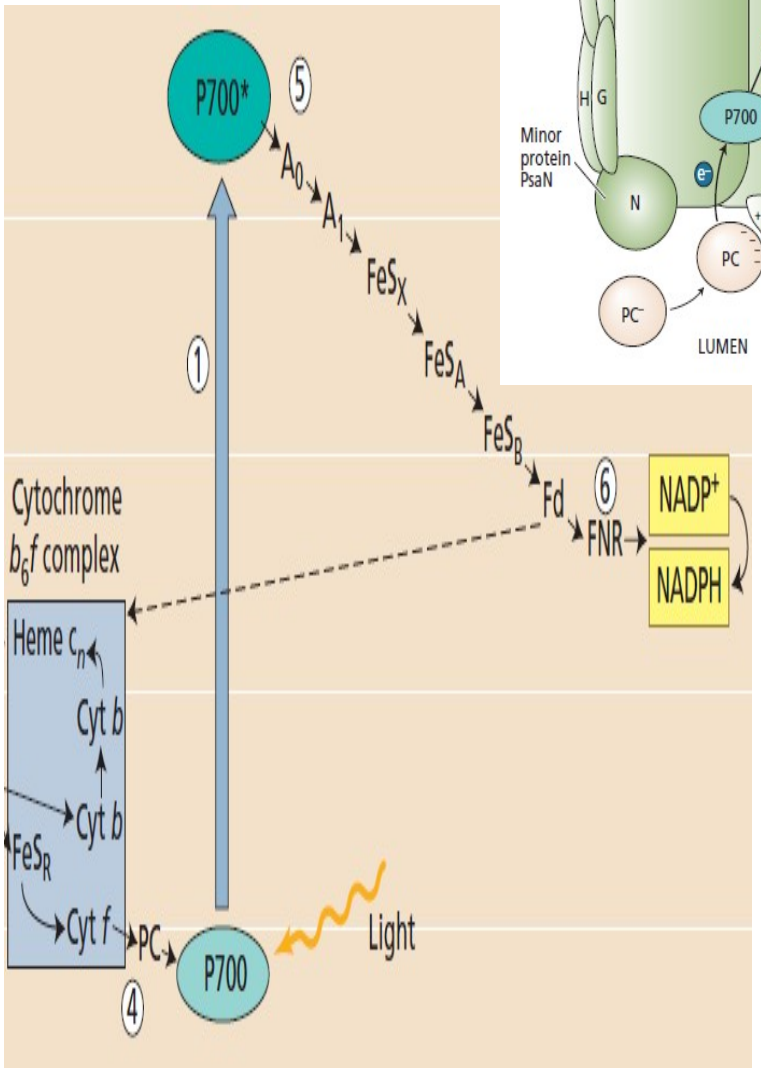
Diffuses in membrane and binds to **Cytochrome b_6F complex** (which mediates **Q-cycle)** where electrons pass from plastoquinol to cytochrome b one at a time

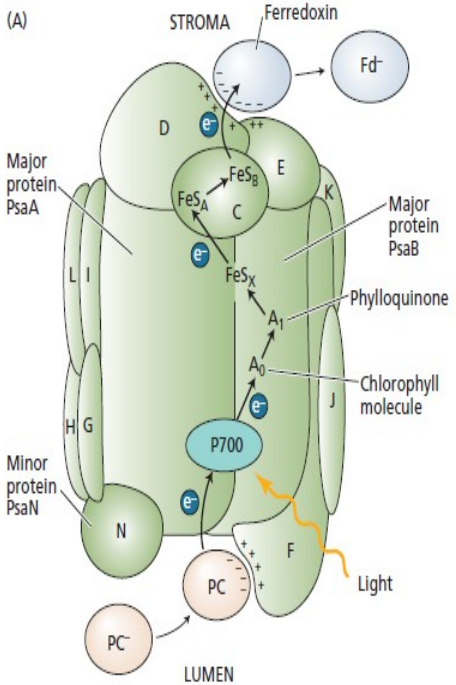
Oxidizes plastoquinol and Pumps protons from stroma into thylakoid lumen











ɔchrome b_6F complex transfers electro

↓

Blue colored, small, water soluble and copper containing protein- **Plastocyanin** (present in lumenal side of thylakoid membrane)

↓

P700 (at core of PSI) absorbs a photon and becomes excited **P700*** accepts 4 electrons

↓

Electron transferred to **A_0** (primary electron acceptor modified **Chl)**

↓

A_1 (Phylloquinone vitamin K1)

↓

Fe-S center (FX, FA, FB); 4Fe-4S

↓

Ferrodoxin (Fd) water soluble, iron-sulfur protein on stromal side

↓

FNR (flavoprotein, Ferrodoxin-$NADP^+$ reductase)

↓

$NADP^+$

- **2e$^-$** →Required for **NADP** for reduction to **NADPH**
- For each **e$^-$** transferred from H_2O to $NADP^+$ = **2 photons** absorbed
- 1 molecule of **O_2** = transfer of **4e$^-$** from **H_2O** to **2 $NADP^+$**

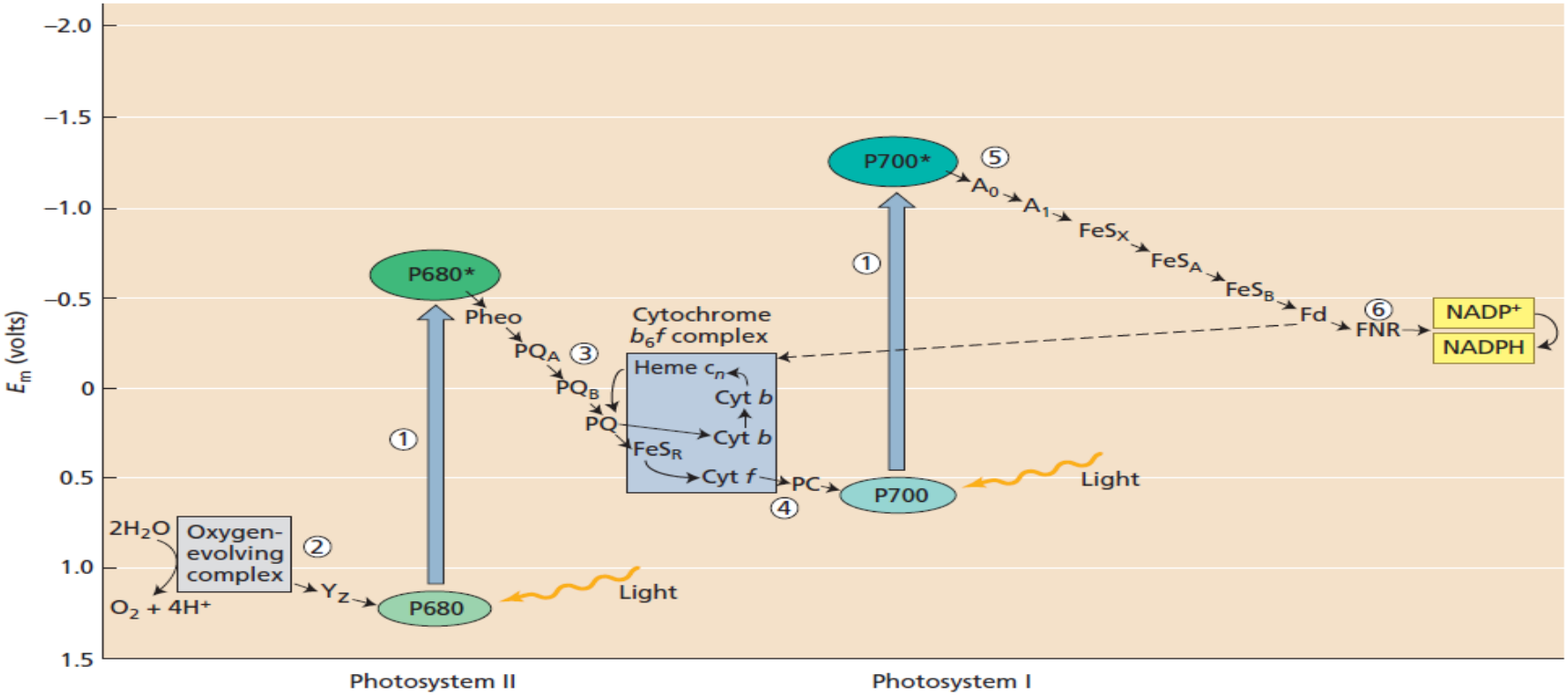


Figure 7.19 Detailed Z scheme for O_2-evolving photosynthetic organisms. The redox carriers are placed at their midpoint redox potentials (at pH 7). (1) The vertical arrows represent photon absorption by the reaction center chlorophylls: P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo). (2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Y_z, which has received electrons from oxidation of water. (3) On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors PQ_A and PQ_B, which are plastoquinones. (4) The cytochrome b_6f complex transfers electrons to plastocyanin (PC), a soluble protein, which in turn reduces P700$^+$ (oxidized P700). (5) The acceptor of electrons from P700* (A_0) is thought to be a chlorophyll, and the next acceptor (A_1) is a quinone. A series of membrane-bound iron–sulfur proteins (FeS_X, FeS_A, and FeS) transfers electrons to soluble ferredoxin (Fd). (6) The soluble flavoprotein ferredoxin–NADP$^+$ reductase (FNR) reduces NADP$^+$ to NADPH, which is used in the Calvin–Benson cycle to reduce CO_2 (see Chapter 8). The dashed line indicates cyclic electron flow around PSI. (After Blankenship and Prince 1985.)

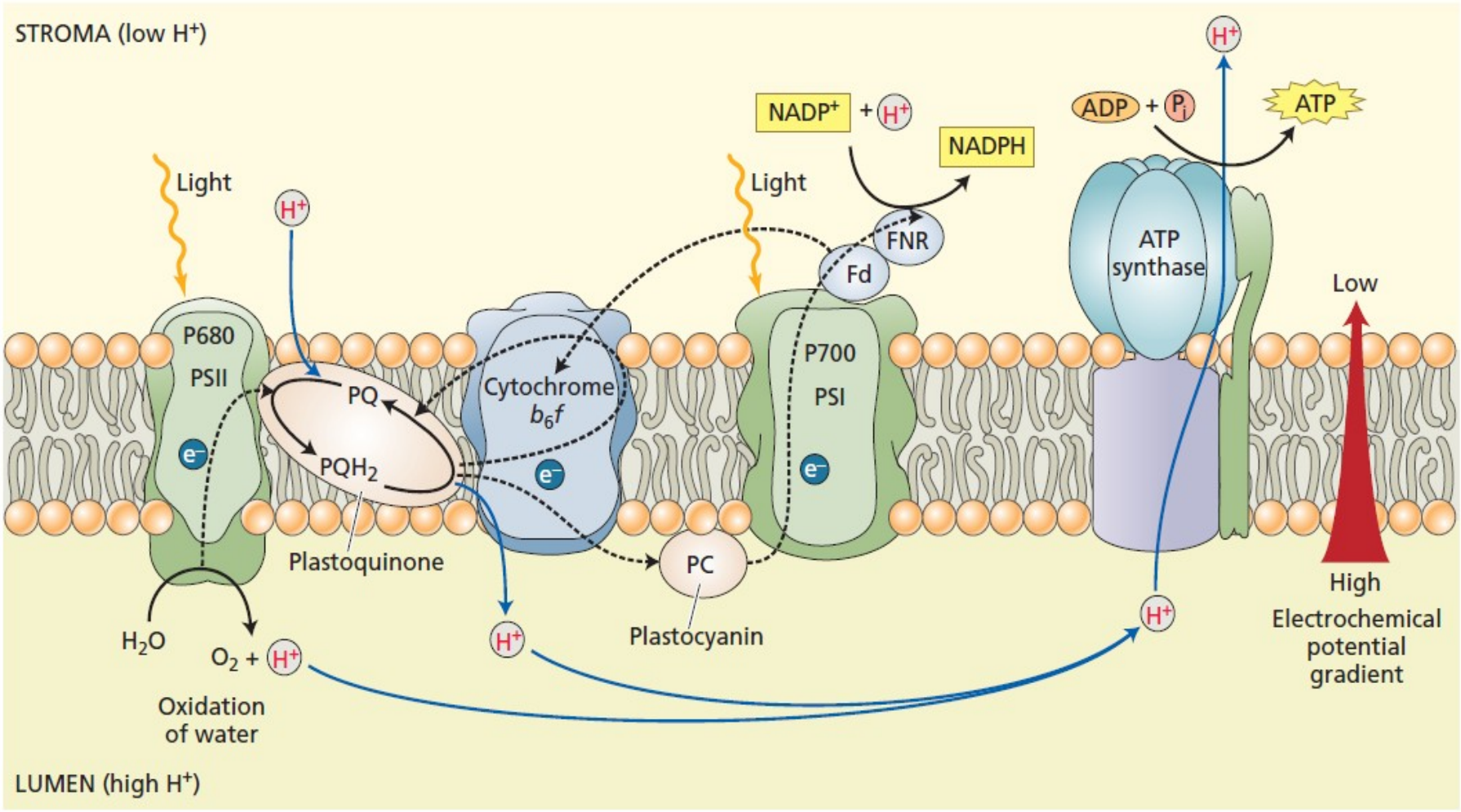


Figure 7.20 Transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes (see Figure 7.16B for structures). Water is oxidized and protons are released in the lumen by PSII. PSI reduces $NADP^+$ to NADPH in the stroma, via the action of ferredoxin (Fd) and the flavoprotein ferredoxin–$NADP^+$ reductase (FNR). Protons are also transported into the lumen by the action of the cytochrome b_6f complex and contribute to the electrochemical proton gradient. These protons must then diffuse to the ATP synthase enzyme, where their diffusion down the electrochemical potential gradient is used to synthesize ATP in the stroma. Reduced plastoquinone (PQH_2) and plastocyanin transfer electrons to cytochrome b_6f and to PSI, respectively. The dashed lines represent electron transfer; solid lines represent proton movement.

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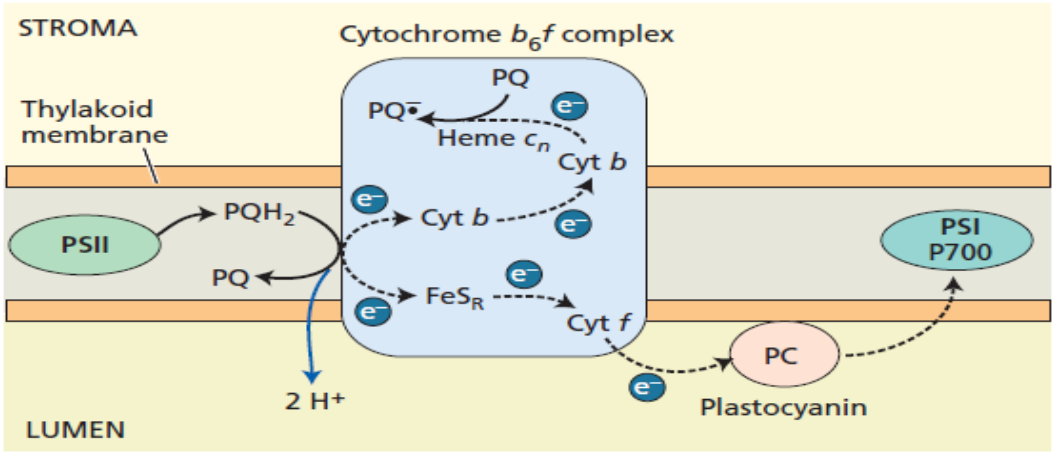
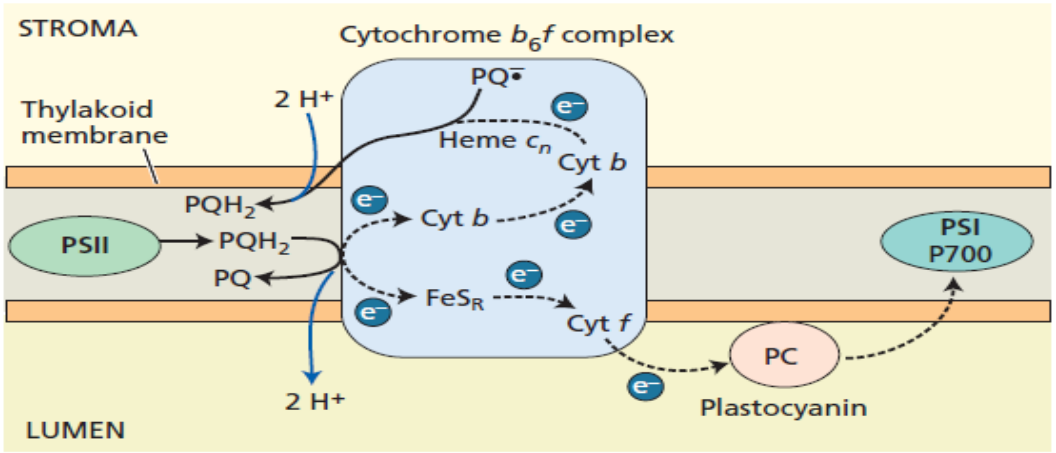
Figure 7.26 Mechanism of electron and proton transfer in the cytochrome b_6f complex. This complex contains two *b*-type cytochromes (Cyt *b*), a *c*-type cytochrome (Cyt *c*, historically called cytochrome *f*), a Rieske Fe–S protein (FeS_R), and two quinone oxidation–reduction sites. (A) The noncyclic or linear processes: A plastohydroquinone (PQH_2) molecule produced by the action of PSII (see Figure 7.24) is oxidized near the lumenal side of the complex, transferring its two electrons to the Rieske Fe–S protein and one of the *b*-type cytochromes and simultaneously expelling two protons to the lumen. The electron transferred to FeS_R is passed to cytochrome *f* (Cyt *f*) and then to plastocyanin (PC), which reduces P700 of PSI. The reduced *b*-type cytochrome transfers an electron to the other *b*-type cytochrome, which reduces a plastoquinone (PQ) to the plastosemiquinone ($PQ^{\bullet}$) state (see Figure 7.24). (B) The cyclic processes: A second PQH_2 is oxidized, with one electron going from FeS_R to PC and finally to P700. The second electron goes through the two *b*-type cytochromes and reduces the plastosemiquinone to the plastohydroquinone, at the same time picking up two protons from the stroma. Overall, four protons are transported across the membrane for every two electrons delivered to P700.

(A) First QH_2 oxidized



(B) Second QH_2 oxidized



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Role of DCMU and Paraquat

1. **DCMU- 3-(3,4 dichlorophenyl)-1,1 Dimethylurea)/ Diuron**
   - Herbicide
   - Abolishes photosynthetic O_2 production
   - Because it competes with Plastoquinone for Q_B binding site of PSII
   - Blocks electron flow from PSII→Cytb_6f complex
2. **Paraquat**
   - Herbicide
   - Blocks photosynthetic electron flow
   - By intercepting electrons between the Ferrodoxin acceptors and $NADP^+$
   - Reduces O_2 → Superoxide (O_2^{*-})
   - Superoxide- free radical that reacts - Nonspecifically with wide range of molecules in

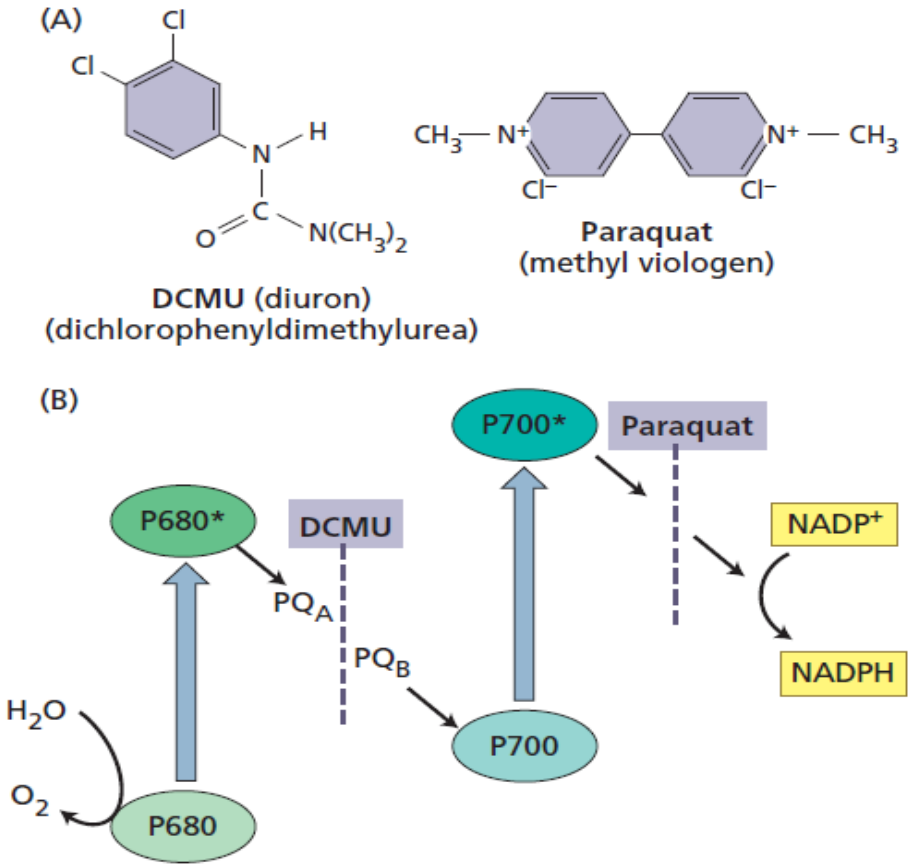

Figure 7.28 Chemical structure and mechanism of action of two important herbicides. (A) Chemical structure of dichlorophenyldimethylurea (DCMU) and methyl viologen (paraquat), two herbicides that block photosynthetic electron flow. DCMU is also known as diuron. (B) Sites of action of the two herbicides. DCMU blocks electron flow at the plastoquinone acceptors of PSII by competing for the binding site of plastoquinone. Paraquat acts by accepting electrons from the early acceptors of PSI.

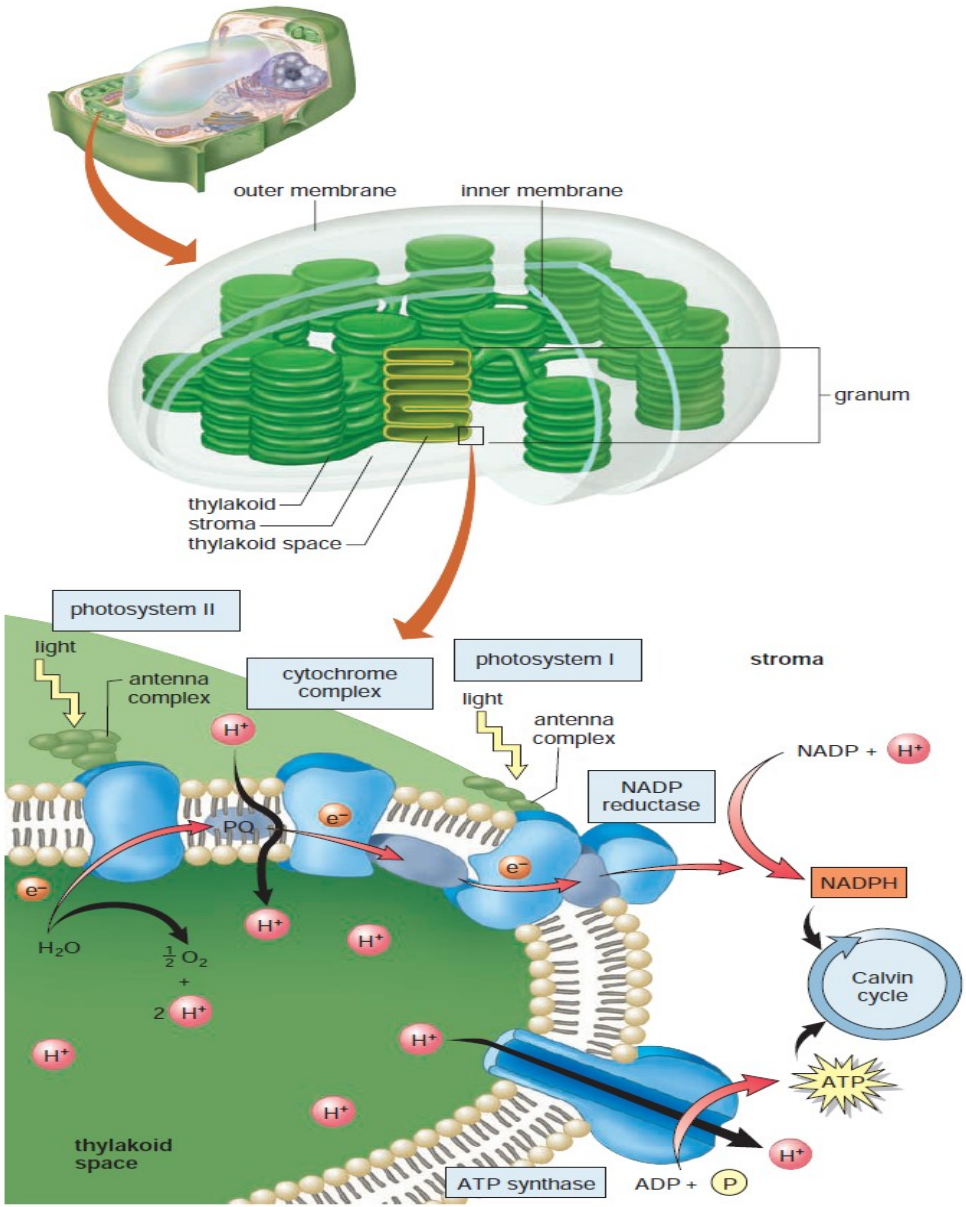

Figure 10.9 Organization of the thylakoid membrane showing the relative positions of photosystems and protein complexes. Some hydrogen ions (protons) from the stroma are pumped into the thylakoid space (lumen), producing a hydrogen gradient. ATP is produced when these hydrogen ions and those from water flow from the thylakoid space into the stroma through the ATP synthase complex. *(Adapted from Sylvia S. Mader, Biology, 7th edition. © 2001 The McGraw-Hill Companies. All rights reserved.)*

ATP synthesis

- Water –electron donor
- Photo oxidation- 2 H_2O = $4e^-$, 4 protons and molecular oxygen→ transported down the electron transport chain ; Energy is released
- Used to pump protons across thylakoid membrane from stroma to thylakoid lumen
- Produces electrochemical proton gradient generates proton motive force
- 8 photos absorption by PSII and PSI
- 4 photons each
- Photo oxidation of 2 H_2O→ 4 protons and Oxygen
- Transport of $4e^-$ through $Cytb_6F$ complex→ translocates 8 Protons from stroma to thylakoid lumen
- Total 12 protons
- Pass back across thylakoid membrane to stroma
- Through ATP synthase (CF_0- CF_1) - Catalyze ATP synthesis
- As protons flow back along electrochemical proton gradient

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Noncyclic electron flow

- Both PSII and PSI
- Photolysis of water
- Formation of Oxygen
- ATP and NADPH synthesis
- Cyclic electron flow
- Only PSI
- No photolysis of water
- No Oxygen formation
- ATP synthesis
- No NADPH

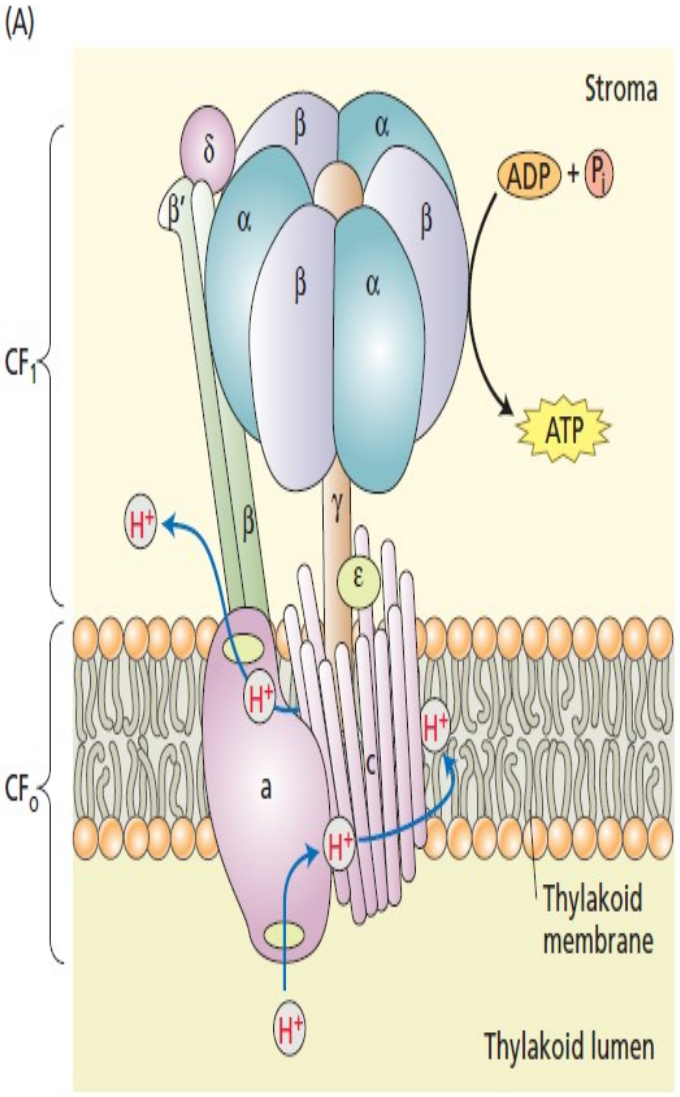
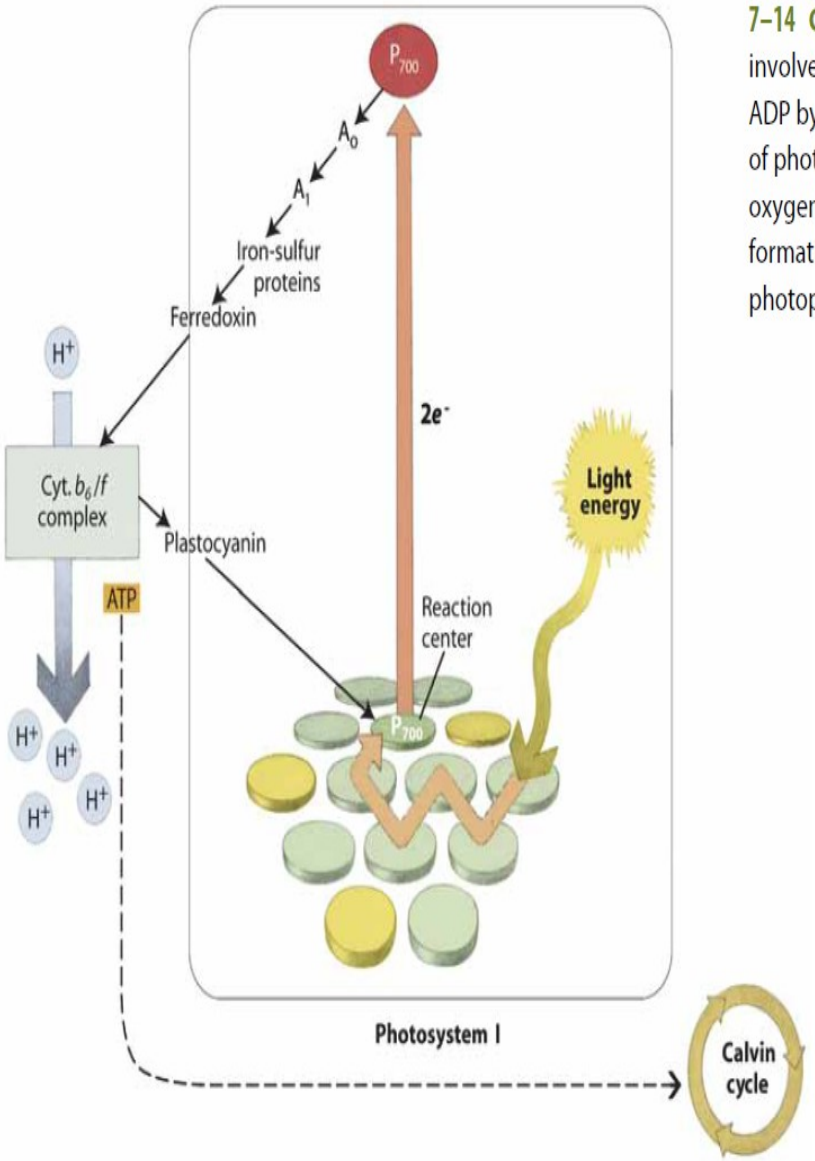


Figure 7.30 Subunit composition (A) and compiled crystal structure (B) of chloroplast F_1F_o ATP synthase. This enzyme consists of a large multi-subunit complex, CF_1, attached on the stromal side of the membrane to an integral membrane portion, known as CF_o. CF_1 consists of five different polypeptides, with stoichiometry of $\alpha_3 \beta_3 \gamma \delta \varepsilon$. CF_o contains probably four different polypeptides, with a stoichiometry of a b b′ c_{14}. Protons from the lumen are transported by the rotating c polypeptide and ejected on the stroma side. The structure closely resembles that of the mitochondrial F_1F_o ATP synthase (see Chapter 12) and the vacuolar V-type ATPase (see Chapter 6). (Figure courtesy of W. Frasch.)

Noncyclic electrons transport
~4 molecules of ATP per molecule of oxygen evolved



7–14 Cyclic electron flow Cyclic electron flow involves only Photosystem I. ATP is produced from ADP by the same chemiosmotic coupling mechanism of photophosphorylation shown in Figure 7–12, but oxygen is not released and $NADP^+$ is not reduced. The formation of ATP by cyclic electron flow is called cyclic photophosphorylation.

Cyclic electron flow

- In some cases, photo excited electrons follow cyclic pathway
- Only **PSI (PS700)** is involved
- P700 transfers electron to **Cytochrome b_6f complex**
- Coupled by proton pumping into thylakoid lumen
- **Cyclic photophosphorylation**
- **No NADPH and Oxygen production**; **only ATP is synthesized**
- Protons flow down the Electrochemical gradient through **ATP synthase complexes**
- Utilized only when NADPH concentration is sufficient but still needs ATP to power other activities in chloroplast
- More productive as compared to non-cyclic photophosphorylation with regard to ATP synthesis
- 4 photons→ PSI = **8 protons** to lumen by Cyt. b-f complex

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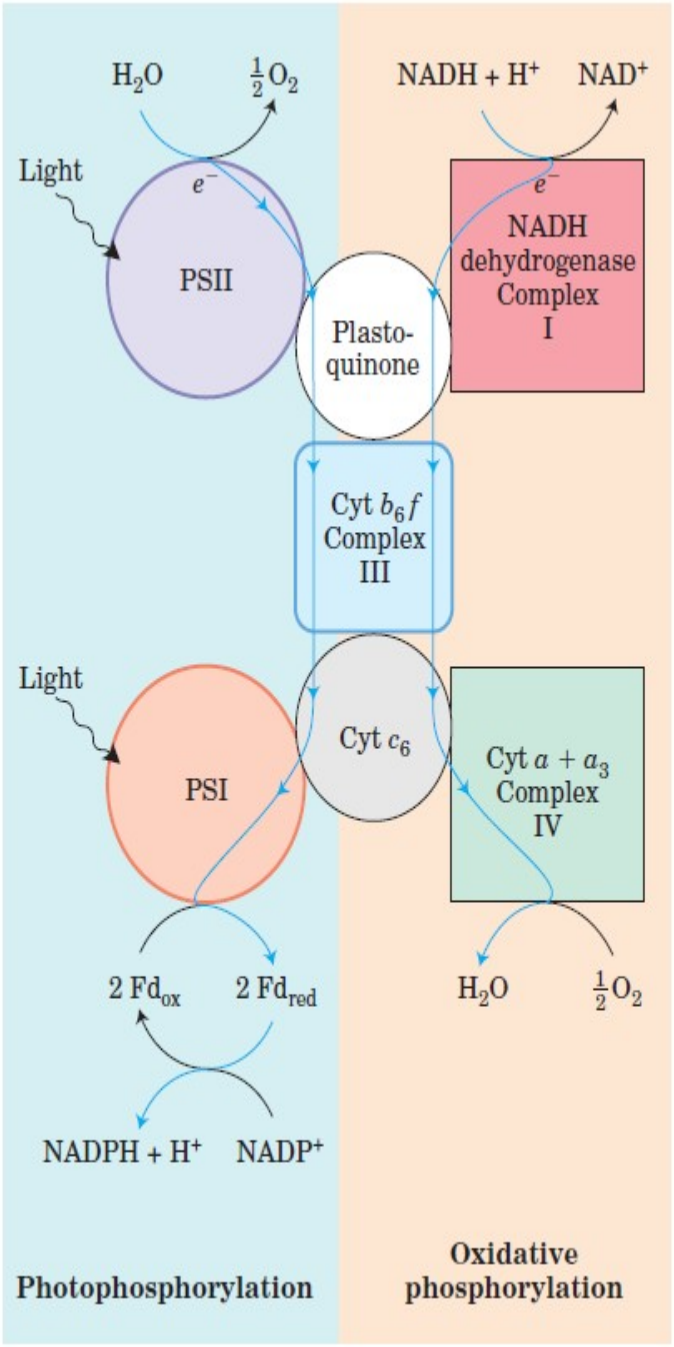
Photophosphorylation

- ATP synthesis driven by light
- Light dependent process
- Occurs during photosynthesis
- In eukaryotes, in chloroplast
- Involves oxidation of **$H_2O \rightarrow O_2$** with **$NADP^+$** as electron acceptor

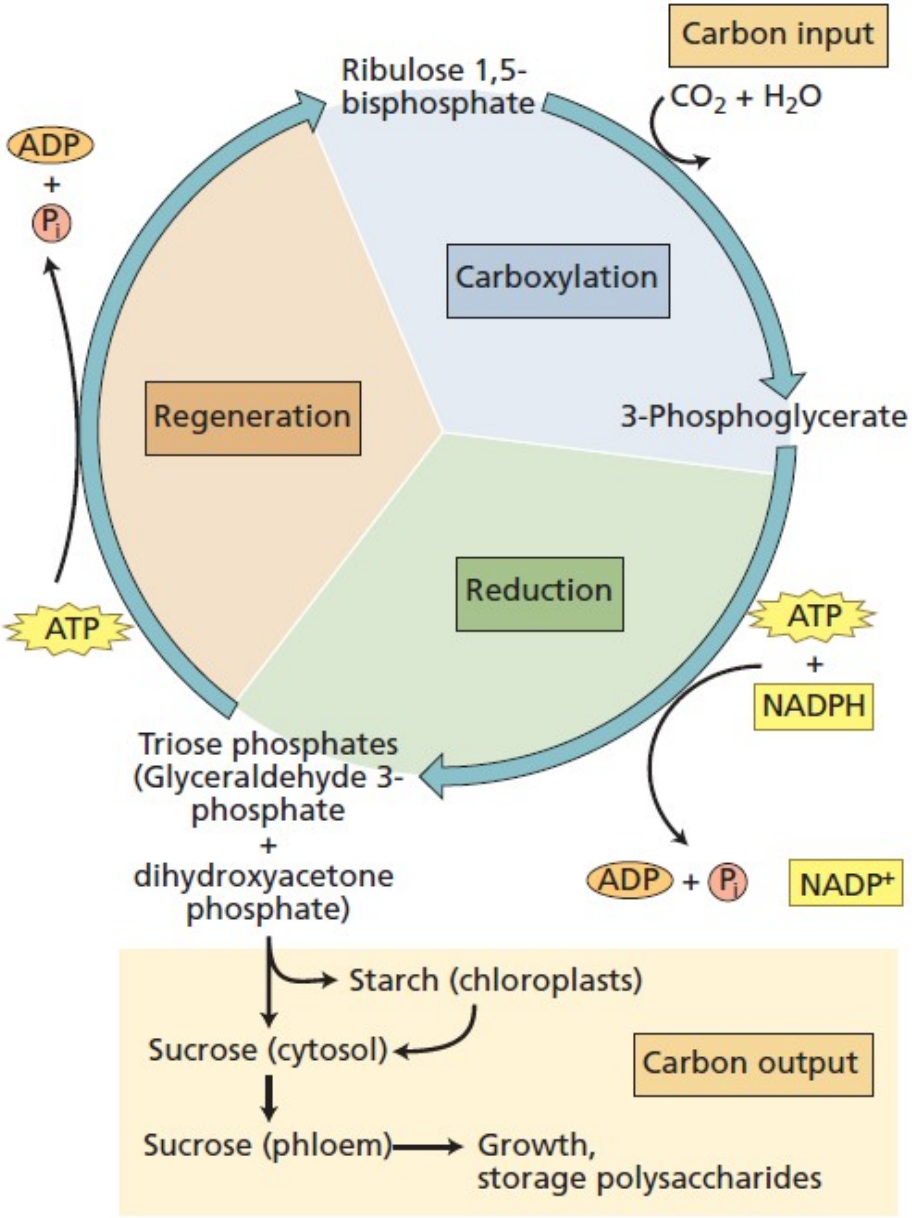


Oxidative photophosphorylation

- ATP synthesis driven by oxidation of **NADH** and **$FADH_2$**
- Light independent process
- Occurs during aerobic respiration
- In eukaryotes, in mitochondria
- Involves reduction of **$O_2 \rightarrow H_2O$**
- With electron donated by **NADH** and **$FADH_2$**

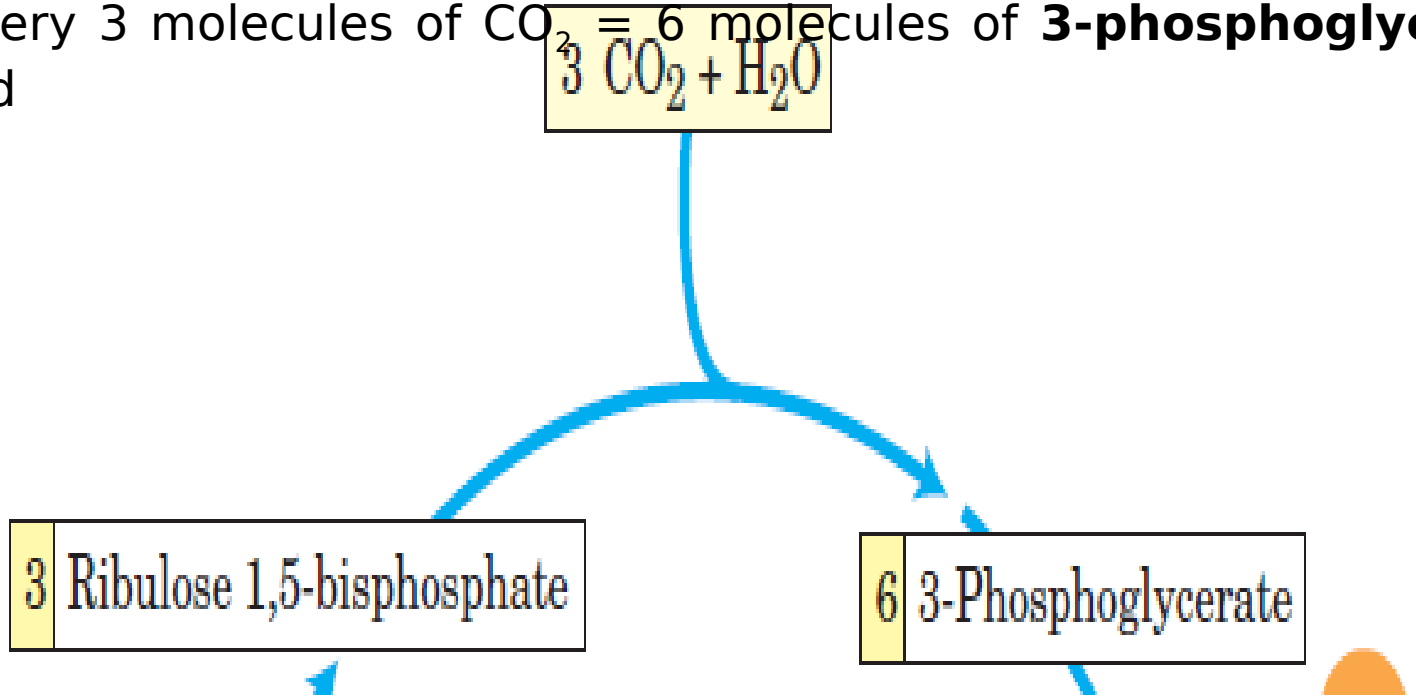
Carbon fixation

- Carbon dioxide reduction and fixation occurs through carbon-fixation cycle called **Calvin cycle**
- Also known as **Reductive pentose phosphate cycle / C_3 cycle**
- Series of reaction which reduces Carbon dioxide to 3 carbon sugar
- Takes place in **Stroma** of chloroplasts in eukaryotic photosynthetic organisms
- Takes place in **Cytosol** in prokaryotic photosynthetic organisms
- Carbohydrate produced directly from the Calvin cycle is actually not glucose but a 3-carbon sugar, **Glyceraldehyde-3-phosphate**
- For net synthesis of one molecule of glyceraldehye-3-phosphate= 3 molecules of CO_2 is needed
- 3 phases of Calvin cycle:
  1. **Carboxylation**
  2. **Reduction**
  3. **Regeneration of the**



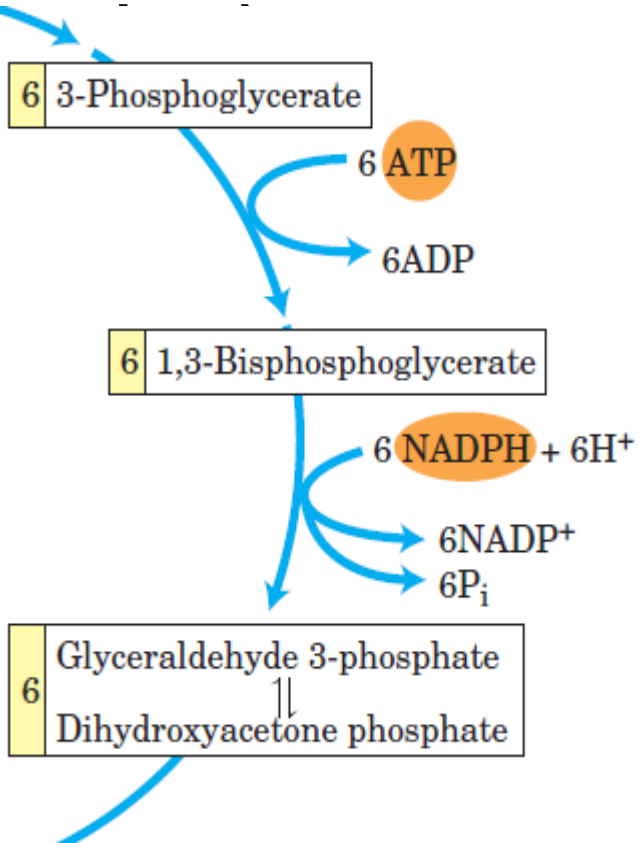
Carboxylation

- CO_2 incorporated into 5-Carbon compound (**Ribulose-1,5-bisphosphate**) **RuBP**
- Enzyme- **Ribulose-1,5-bisphophate carboxylase/ oxygenase (RuBisCo)**
- Product = 6- Carbon intermediate (immediately splits to form 2 molecules of 3-phosphoglycerate (also termed as **Glycerate-3-phosphate**)
- For every 3 molecules of CO_2 = 6 molecules of **3-phosphoglycerate** formed



Reduction

- Reduction of 3-phosphoglycerate into **Glyceraldehyde-3-phosphate**
- First, 3-phosphoglycerate is phosphorylated at the expense of 1ATP to form **1,3-bisphosphoglycerate** (enzyme= **phosphoglycerate kinase**)
- Then, reduced by **NADP: glyceraldehyde-3-phosphate dehydrogenase** to form 1 molecule of **Glyceraldehyde-3-phosphate**
- **6 ATP** utilized = to phosphorylate **6 (3-phosphoglycerate)** to **6 (1,3-bisphosphoglycerate)**
- **6NADPH** consumed= to reduce **6 (1,3-bisphosphoglycerate)** to **6 (glyceraldehyde-3-**



Regeneration

- The net production of fixed carbon in Calvin cycle = 1 (**Glyceraldehyde-3-phosphate**)
- Other 5 glyceraldehylde-3-phosphate- processed in remainder of the Calvin cycle
- Regeneration of 3 molecules of RuBP
- Accomplished by series of reactions
- Intermediates in this pathway include three-, four-, five-, six- and seven- carbon sugars

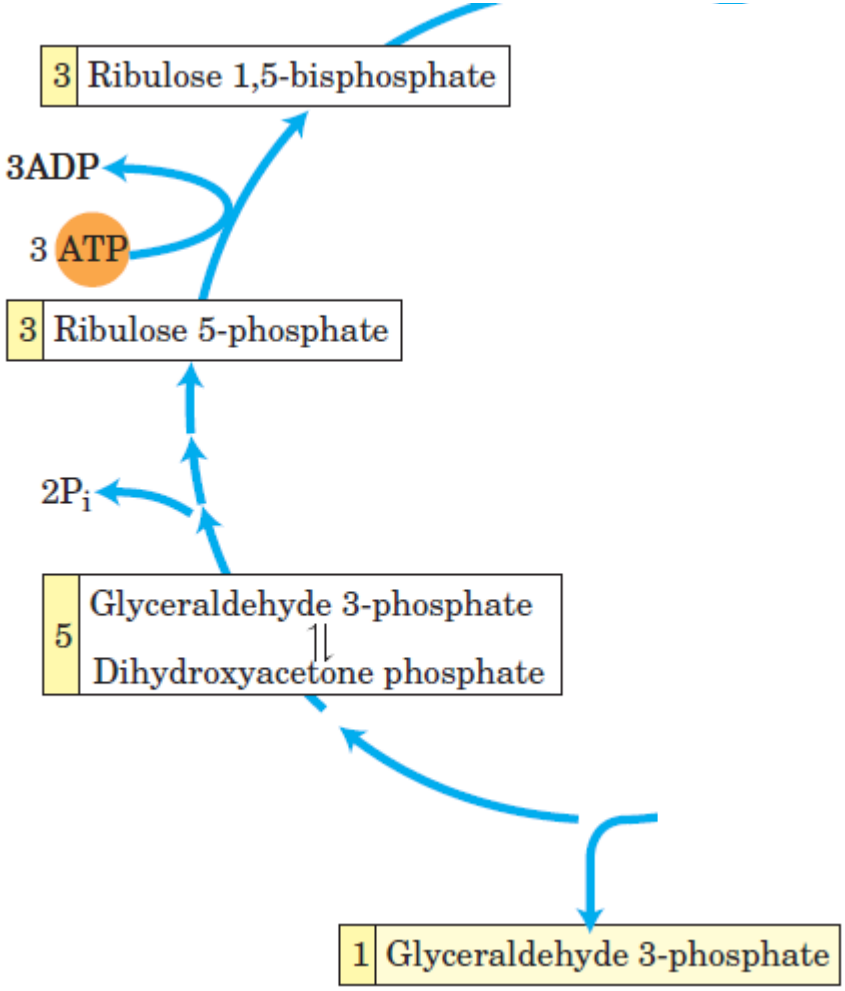
$C_3 + C_3 \rightarrow C_6$

$C_3 + C_6 \rightarrow C_5 + C_4$

$C_3 + C_4 \rightarrow C_7$

$C_3 + C_7 \rightarrow C_5 + C_5$

Overall $5C_3 \rightarrow 3C_5$



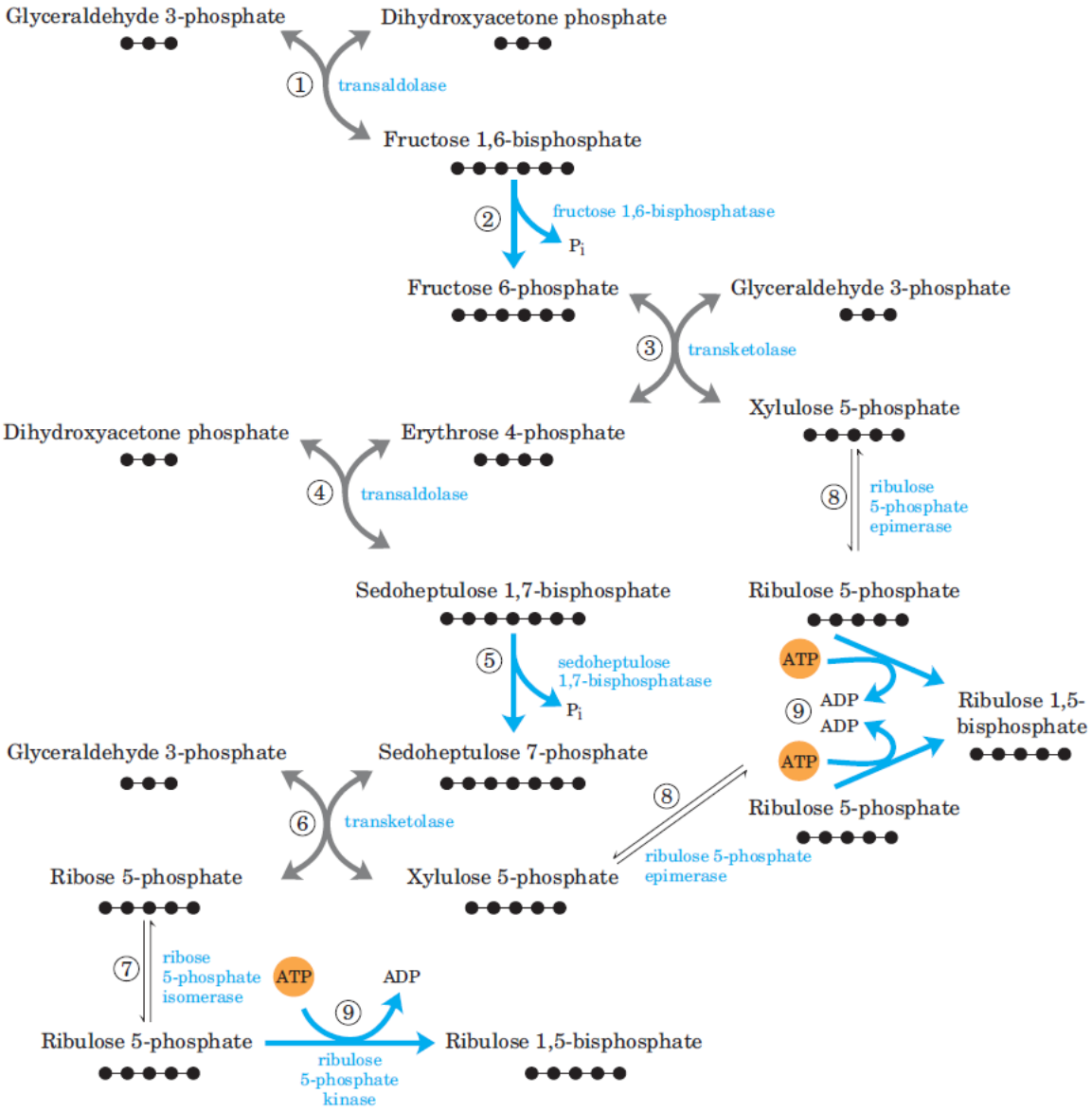


FIGURE 20–10 **Third stage of CO_2 assimilation.** This schematic diagram shows the interconversions of triose phosphates and pentose phosphates. Black dots represent the number of carbons in each compound. The starting materials are glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Reactions catalyzed by transaldolase (① and ④) and transketolase (③ and ⑥) produce pentose phosphates that are converted to ribulose 1,5-bisphosphate—ribose 5-phosphate by ribose 5-phosphate isomerase (⑦) and xylulose 5-phosphate by ribulose 5-phosphate epimerase (⑧). In step ⑨, ribulose 5-phosphate is phosphorylated, regenerating ribulose 1,5-bisphosphate. The steps with blue arrows are exergonic and make the whole process irreversible: steps ② fructose 1,6-bisphosphatase, ⑤ sedoheptulose bisphosphatase, and ⑨ ribulose 5-phosphate kinase.

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TABLE 8.1 Reactions of the Calvin–Benson cycle

Enzyme	Reaction
1. Ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco)	Ribulose 1,5-bisphosphate + CO_2 + H_2O → 2 3-phosphoglycerate
2. 3-Phosphoglycerate kinase	3-Phosphoglycerate + ATP → 1,3-bisphosphoglycerate + ADP
3. NADP–glyceraldehyde-3-phosphate dehydrogenase	1,3-Bisphosphoglycerate + NADPH + H^+ → glyceraldehyde 3-phosphate + $NADP^+$ + P_i
4. Triose phosphate isomerase	Glyceraldehyde 3-phosphate → dihydroxyacetone phosphate
5. Aldolase	Glyceraldehyde 3-phosphate + dihydroxyacetone phosphate → fructose 1,6-bisphosphate
6. Fructose 1,6-bisphosphatase	Fructose 1,6-bisphosphate + H_2O → fructose 6-phosphate + P_i
7. Transketolase	Fructose 6-phosphate + glyceraldehyde 3-phosphate → erythrose 4-phosphate + xylulose 5-phosphate
8. Aldolase	Erythrose 4-phosphate + dihydroxyacetone phosphate → sedoheptulose 1,7-bisphosphate
9. Sedoheptulose 1,7-bisphosphatase	Sedoheptulose 1,7-bisphosphate + H_2O → sedoheptulose 7-phosphate + P_i
10. Transketolase	Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate → ribose 5-phosphate + xylulose 5-phosphate
11a. Ribulose 5-phosphate epimerase	Xylulose 5-phosphate → ribulose 5-phosphate
11b. Ribose 5-phosphate isomerase	Ribose 5-phosphate → ribulose 5-phosphate
12. Phosphoribulokinase (ribulose 5-phosphate kinase)	Ribulose 5-phosphate + ATP → ribulose 1,5-bisphosphate + ADP + H^+

Note: P_i stands for inorganic phosphate.

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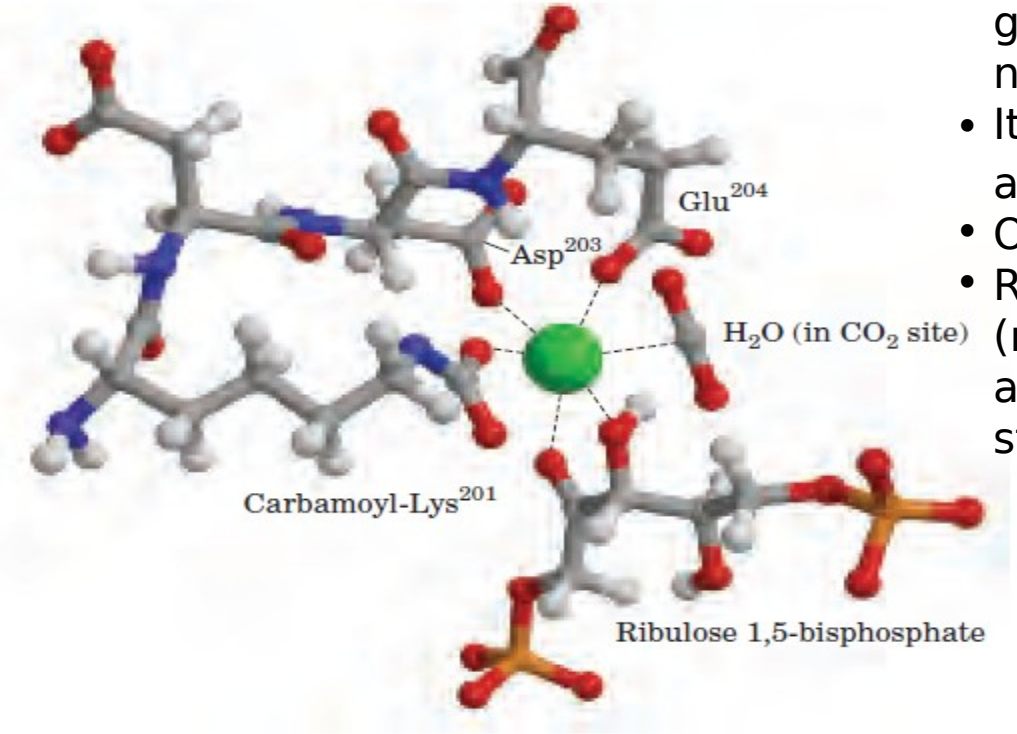
Control of Calvin cycle

- It occurs during day time (Does not occur literally in the dark (at night))
- The metabolic activity at night is mainly shifted to carbohydrate oxidation when plants consume oxygen rather than producing it
- Strictly dependent on the products of light reaction (**NADPH** and **ATP**)
- Control of Calvin cycle = Control



Regulation of enzymes involved in carboxylation:

- Enzymes are pH sensitive

RuBisCo

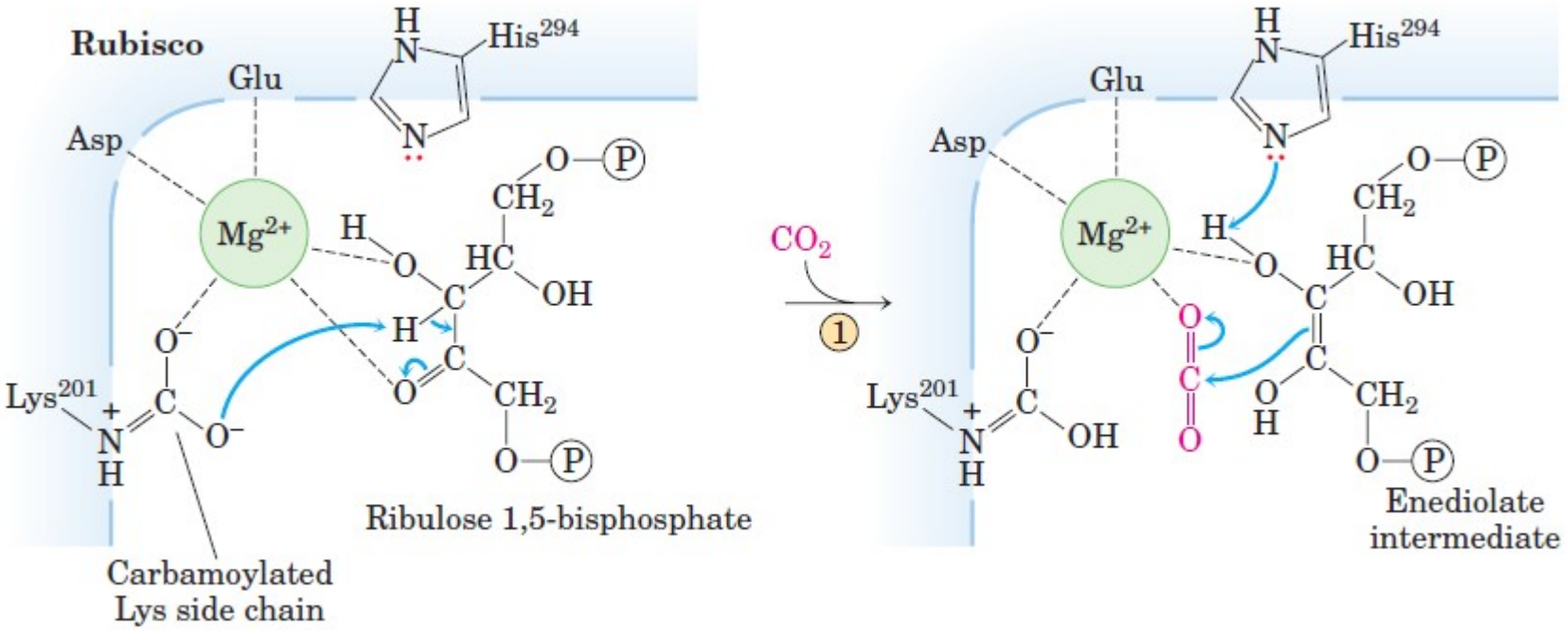
- Pacemaker enzyme of Calvin cycle
- Most abundant proteins in chloroplasts
- Multisubunit enzyme (composed of 8 larger, 8 small subunits)
- Large subunit- encoded by chloroplast genome; small subunit- encoded by nuclear genome
- Its activity is regulated by CO_2, O_2, Mg^{2+} and pH
- Optimum pH for RuBisCo activity is pH 8
- RuBisCo needs bound divalent metal ion (magnesium ion) – this serves to activate a bound substrate molecule by stabilizing a negative charge

FIGURE 20–6 Central role of Mg^{2+} in the catalytic

mechanism of rubisco. (Derived from PDB ID 1RXO.) Mg^{2+} is coordinated in an octahedral complex with six oxygen atoms: one oxygen in the carbamate on Lys^{201}; two in the carboxyl groups of Glu^{204} and Asp^{203}; two at C-2 and C-3 of the substrate, ribulose 1,5-bisphosphate; and one in the other substrate, CO_2. A water molecule occupies the Co2–binding site in this crystal structure. (Residue numbers refer to the spinach enzyme.)

Beginning of Calvin cycle= Conversion of RuBP into highly reactive enediolate intermediate

CO_2 molecule combines with enediolate intermediate = unstable 6-carbon compound (rapidly hydrolyzed to 2 molecules of 3-phosphoglycerate)



1 H_2O is then added to this β –ketoacid to form an intermediate that cleaves to form 2 molecules of 3-phosphoglycerate

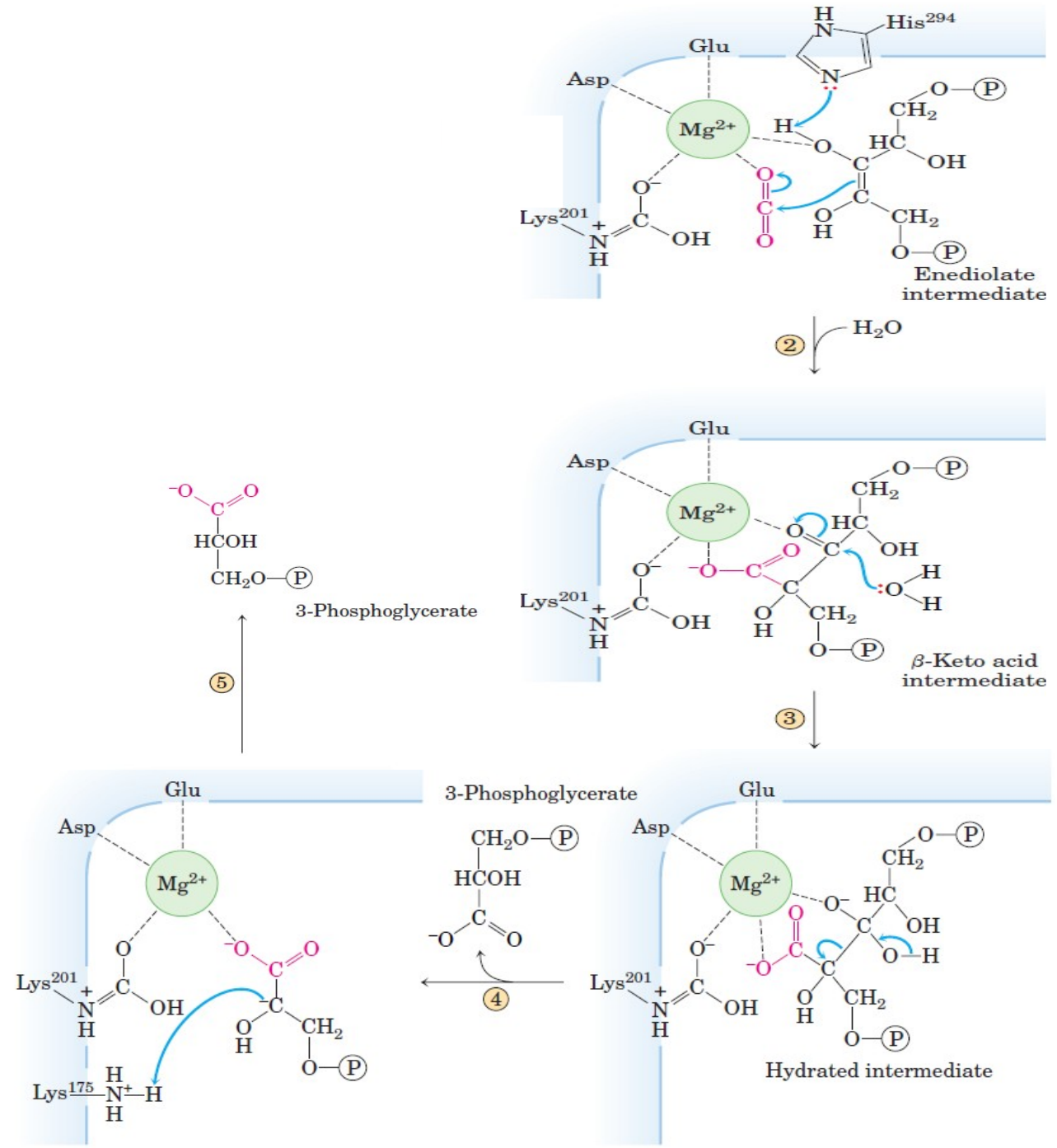
CO_2 molecule other than the substrate is required to complete the assembly of the Mg^{2+} binding site of RuBisCO

This CO_2 molecule combines with the ϵ -amino group of lysine^{201} of large subunits of RuBisCo to form a carbamate

This negatively charged adduct then binds the Mg^{2+} ion

The complex is readily deprotonated to form an enediolate intermediate

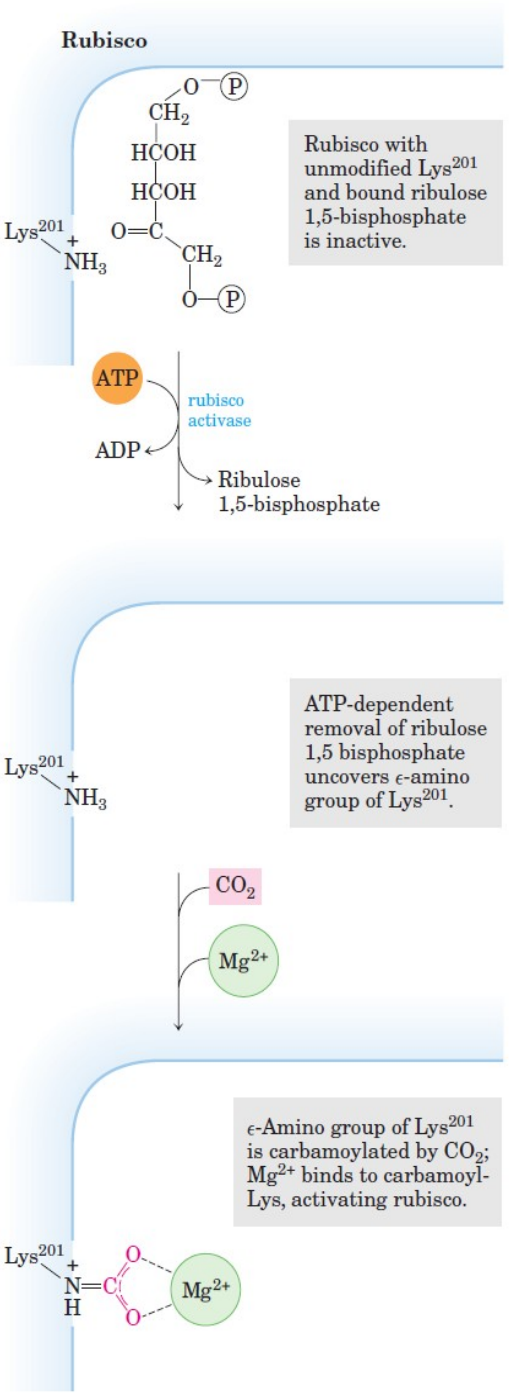
This reactive species couples with CO_2 and forms 2-carboxy-3-keto-D-arabinitol 1,5-bisphosphate



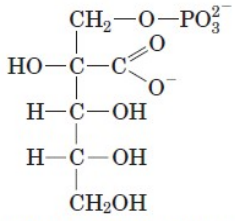
- In presence of light,
- Protons pumped stroma → lumen of thylakoid
- Proton efflux = coupled to magnesium uptake into the stroma
- Ion fluxes = decrease stromal concentration of proton(increases stromal pH 7-8) and increase that of magnesium
- Magnesium stabilizes carbamate formation
- **High pH = increased magnesium concentration= activation of RuBisCo = activation of Calvin cycle**

- RuBisCO= regulated by natural sugar phosphate (2-carboxyarabinitol-1-phosphate (nocturnal inhibitor)
- Has structure similar to that of β–keto acid intermediate of the RuBisCo catalyzed reaction

FIGURE 20–8 **Role of rubisco activase in the carbamoylation of Lys^{201} of rubisco.** When the substrate ribulose 1,5-bisphosphate is bound to the active site, Lys^{201} is not accessible. Rubisco activase couples ATP hydrolysis to expulsion of the bound sugar bisphosphate, exposing Lys^{201}; this Lys residue can now be carbamoylated with CO_2 in a reaction that is apparently not enzyme-mediated. Mg^{2+} is attracted to and binds to the negatively charged carbamoyl-Lys, and the enzyme is thus activated.


Rubisco
Rubisco with unmodified Lys^{201} and bound ribulose 1,5-bisphosphate is inactive.
ATP
rubisco activase
ADP
Ribulose 1,5-bisphosphate
ATP-dependent removal of ribulose 1,5 bisphosphate uncovers ϵ-amino group of Lys^{201}.
CO_2
Mg^{2+}
ϵ-Amino group of Lys^{201} is carbamoylated by CO_2; Mg^{2+} binds to carbamoyl-Lys, activating rubisco.

- **RuBisCo activase-** enzyme essential for RuBisCo activity (it uses ATP to induce structural changes in RuBisCo) that allow release of the bound RuBP and formation of the required carbamate


2-Carboxyarabinitol 1-phosphate

Regulation of enzymes involved in RuBP regeneration:

- Key enzyme- **Fructose bisphosphatase and Sedoheptulose bisphosphatase** –more active at pH 8, require magnesium as a cofactor for catalysis
- Sensitive to redox potential of chloroplast stromal compartment
- Bisphosphatases are active in their reduced state only sensing the oxidation state of **PSI**

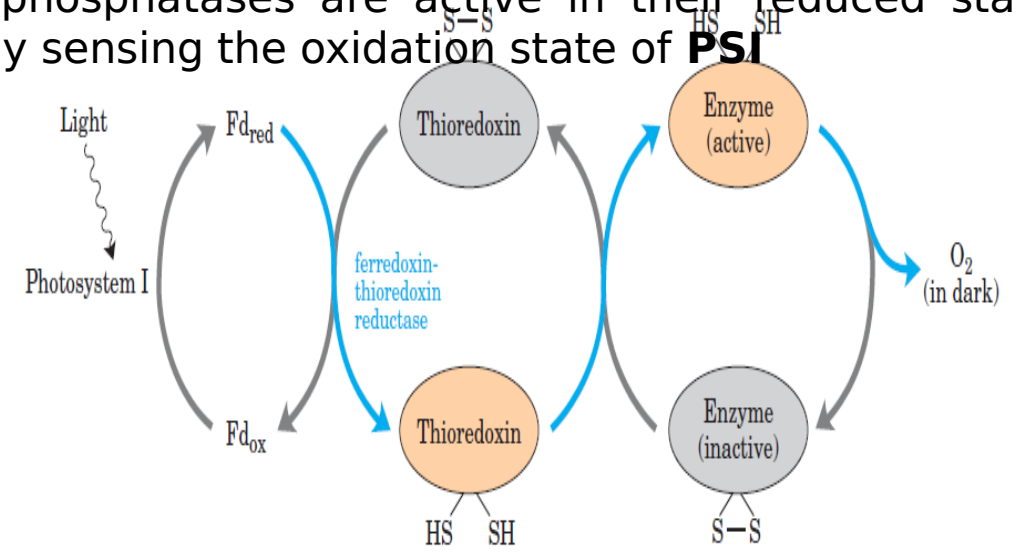


FIGURE 20-19 Light activation of several enzymes of the Calvin cycle. The light activation is mediated by thioredoxin, a small, disulfide-containing protein. In the light, thioredoxin is reduced by electrons moving from photosystem I through ferredoxin (Fd) (blue arrows), then thioredoxin reduces critical disulfide bonds in each of the enzymes sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphosphatase, ribulose 5-phosphate kinase, and glyceraldehye 3-phosphate dehydrogenase, activating these enzymes. In the dark, the —SH groups undergo reoxidation to disulfides, inactivating the enzymes.

- Mediator molecules- **thioredoxin** (is coupled to Ferrodoxin by **Ferrodoxin-thioredoxin reductase**)
- Inhibits phosphofructokinase
- In plants, light stimulates the Calvin cycle enzymes (PSI being the strong reductant) and inhibits glycolysis
- Light activation mediated by thioredoxin- in light, thioredoxin reduced by electrons moving from PSI through **Ferrodoxin** (**Fd**), then thioredoxin reduces critical disulfide bonds in enzymes such as **Sedoheptulose 1,7-bisphosphatase** and **Fructose 1,6-bisphosphatase**,

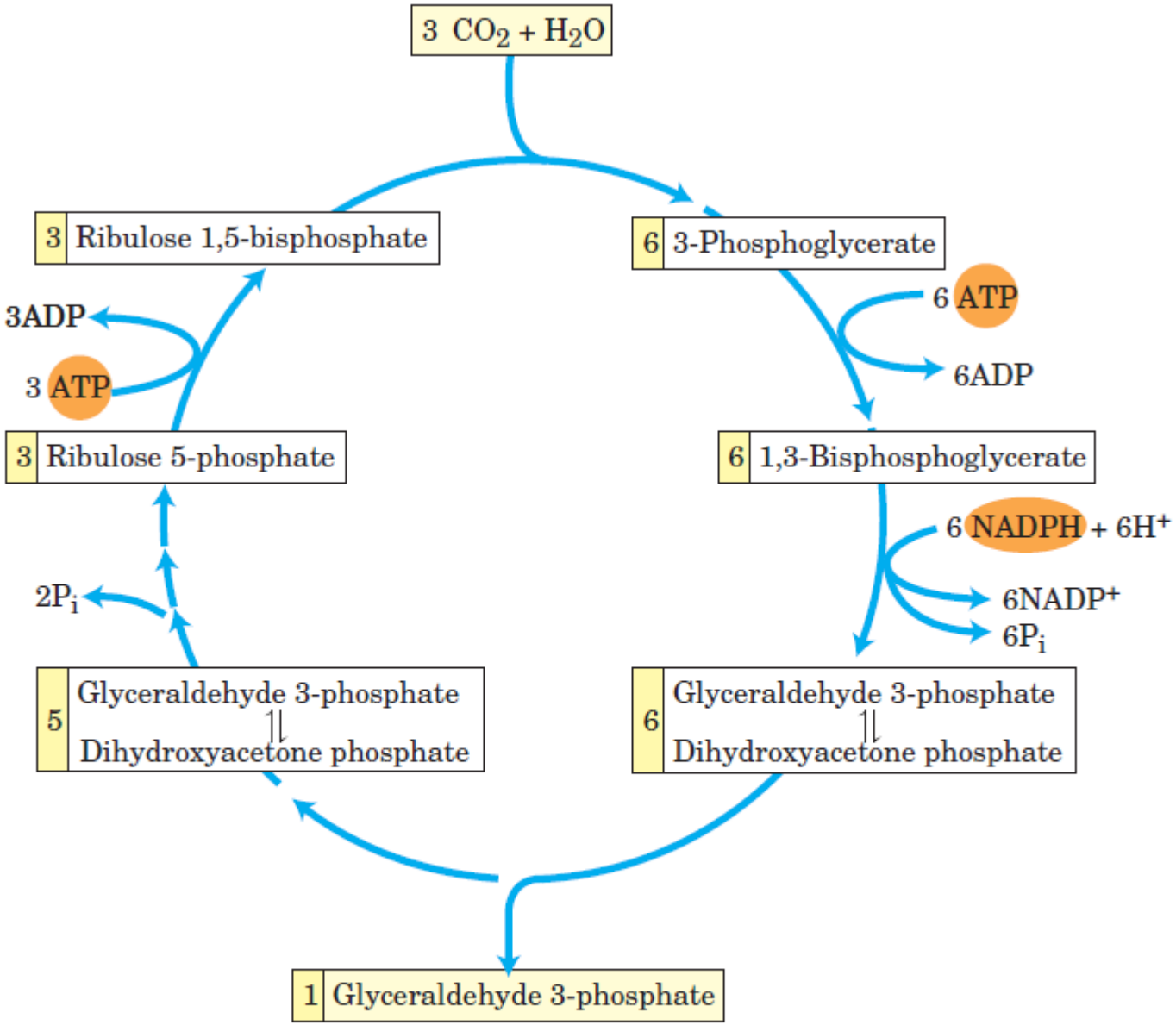
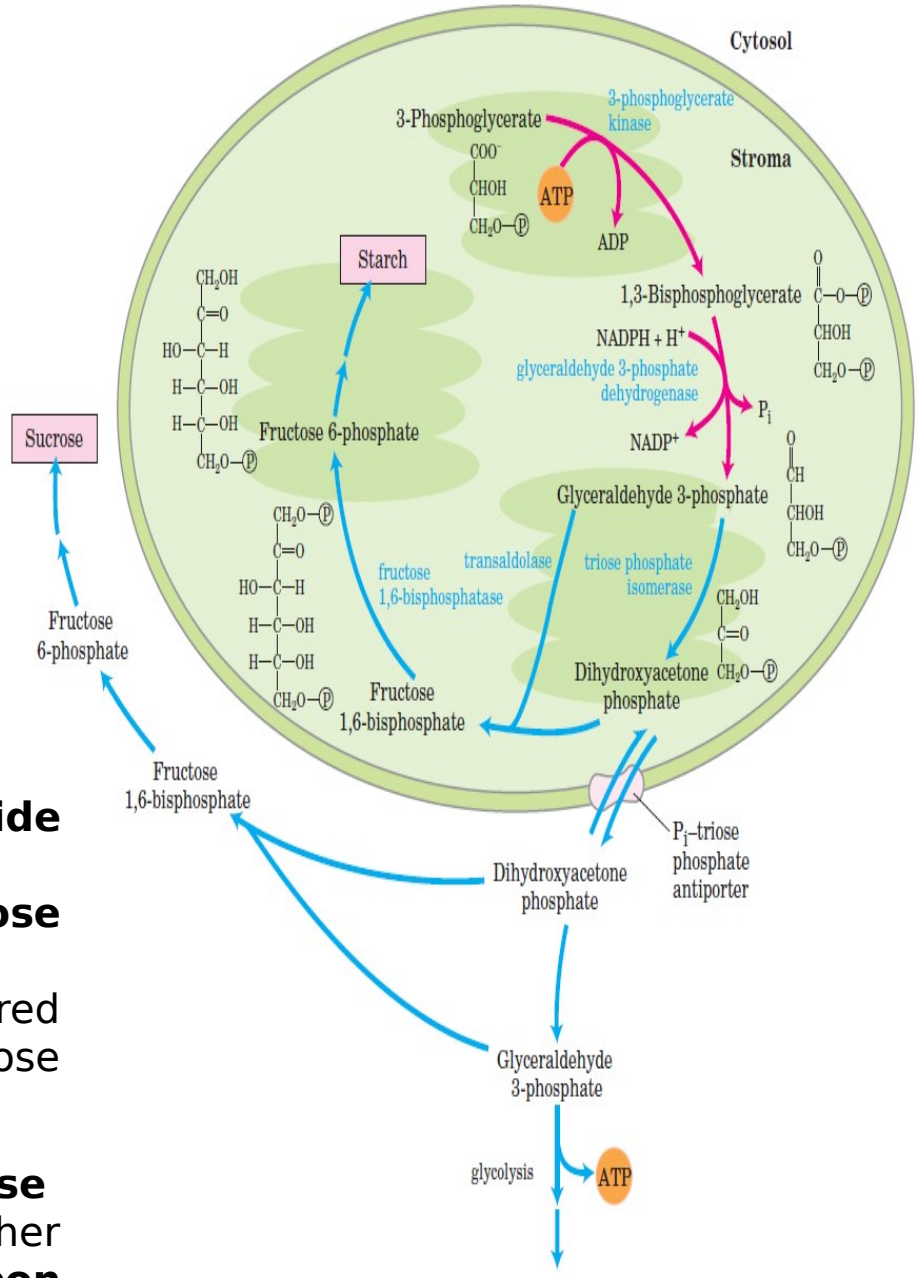


FIGURE 20–14 Stoichiometry of CO_2 assimilation in the Calvin cycle. For every three CO_2 molecules fixed, one molecule of triose phosphate (glyceraldehyde 3-phosphate) is produced and nine ATP and six NADPH are consumed.

Rashmi MC

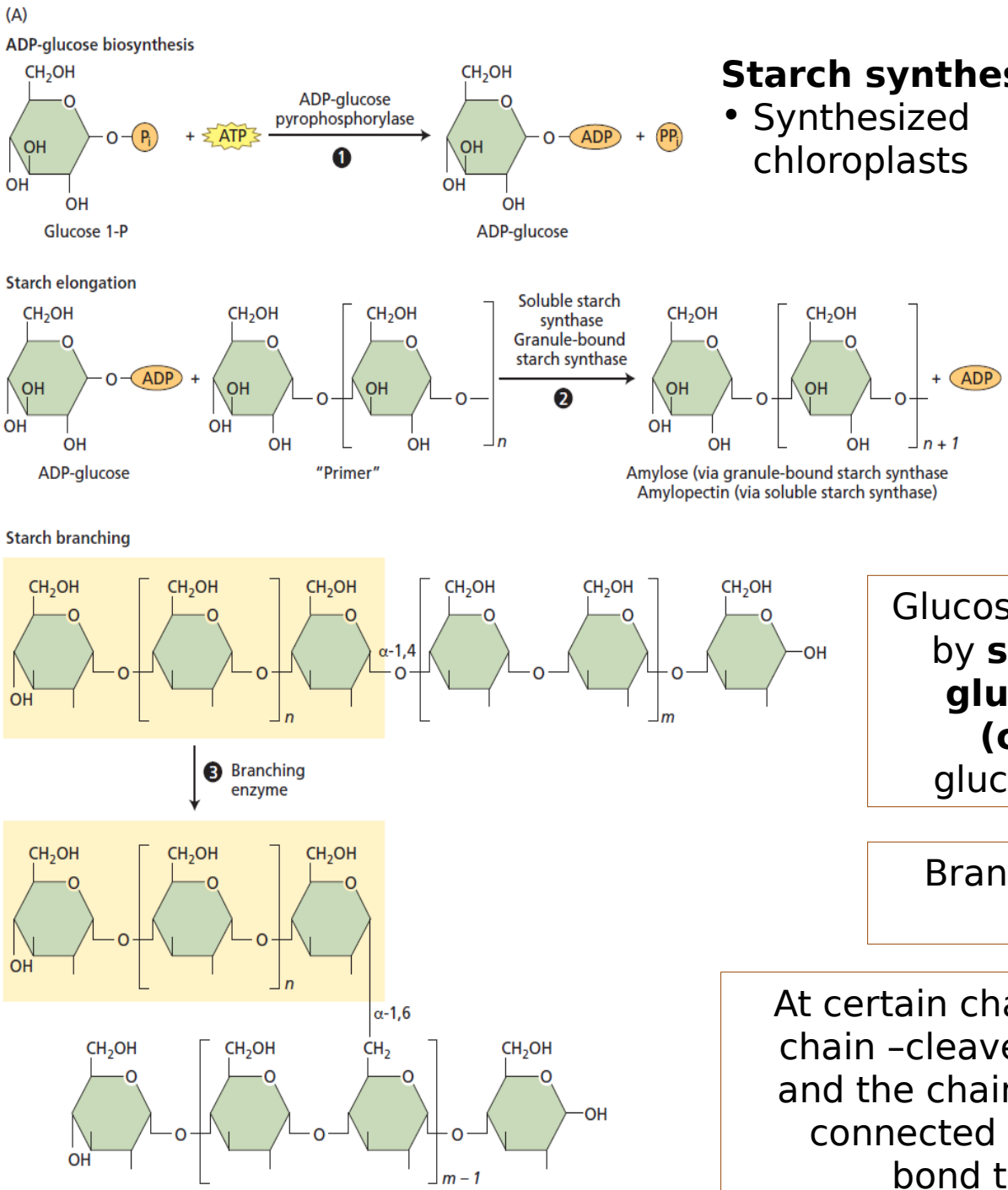
Starch and sucrose synthesis

- Precursor for synthesis of both starch and sucrose = **Triose phosphate (Glyceraldehyde-3-phosphate)**, generated by Calvin cycle
- **Sucrose- Transportable form of carbon**
- **Starch- Storage form of carbon**
- Synthesis of starch and sucrose- competing processes that occur in **Chloroplast** and **Cytosol** respectively
- When,
  - **Cytosolic P_i concentration is high = chloroplast triose phosphate- exported to cytosol = sucrose is synthesized**
  - **Cytosolic P_i concentration is low = triose phosphate is retained within the chloroplast = starch is synthesized**



- **During starch synthesis,**
- Glucose residue is activated as **Nucleoside Sugar**
- **Triose phosphate** converted to **ADP-glucose** –via Fructose-1,6-bisphosphate
- Glucose moiety of ADP- glucose is transferred to the non-reducing end of the terminal glucose of a growing starch chain

- **During Sucrose synthesis,**
- **Triose phosphate** converted to **UDP-glucose**
- Conversion of the photoassimilates to either sucrose or to starch is called **Carbon Allocation**



Starch synthesis

- Synthesized from triose phosphate in chloroplasts

Triose phosphate converted into **Glucose-1-phosphate**

↓

Glucose-1-phosphate activated to **ADP-glucose** (Enzyme- **ADP-glucose pyrophosphorylase**)

↓

Glucose residue- finally transferred by **starch synthase** from **ADP-glucose** to **nonreducing end (carbon 4)** of the terminal glucose of growing starch chain

↓

Branches formed- by branching enzyme

↓

At certain chain lengths, polysaccharide chain –cleaved at (α1→4) glycosidic bond and the chain fragment thus separated is connected via a newly formed (α1→6) bond to a neighboring chain

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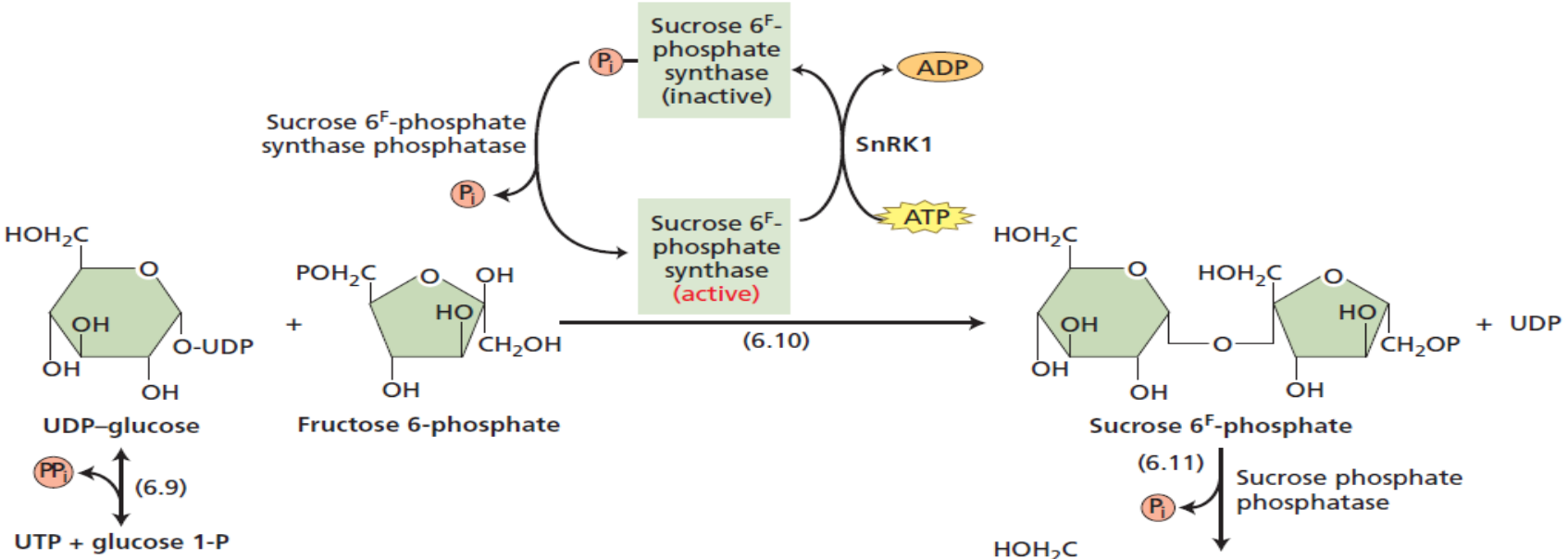

Sucrose synthesis

- Disaccharide of glucose and fructose forms sucrose that takes place in cytosol
- Synthesized in cytosol from triose phosphates

Triose phosphate converted into **Glucose-1-phosphate**

→

Glucose-1-phosphate is activated to **UDP-glucose** (Enzyme- **UDP-glucose pyrophosphorylase**)

↓

Transfer of the glucose residue from UDP-glucose to fructose-6-phosphate forming sucrose-6-phosphate (Enzyme- **Sucrose phosphate synthase)**

←

Cleavage of phosphate from **Sucrose -6 phosphate** yielding sucrose (Enzyme - **Sucrose-6-phosphate phosphatase**)

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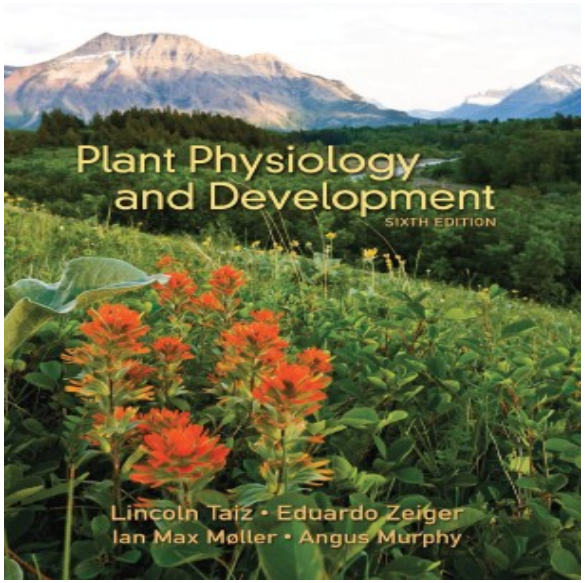
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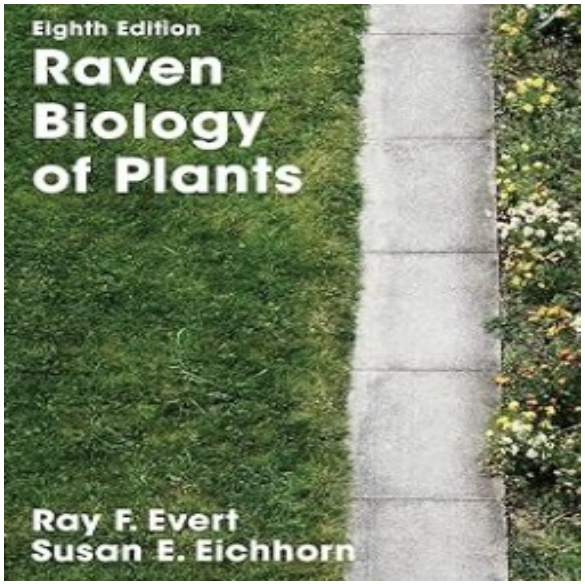
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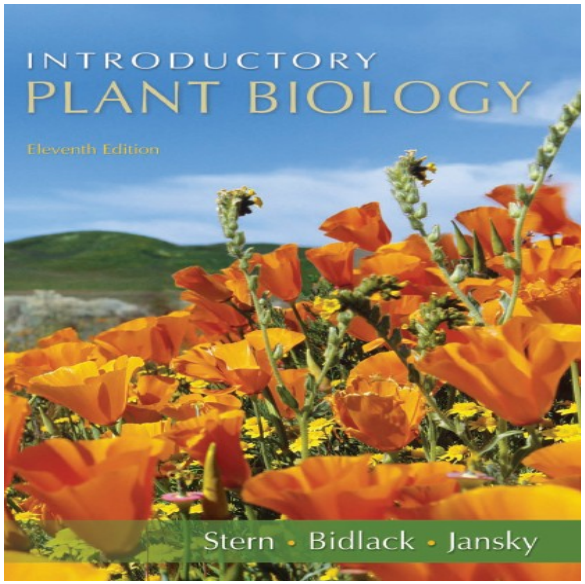
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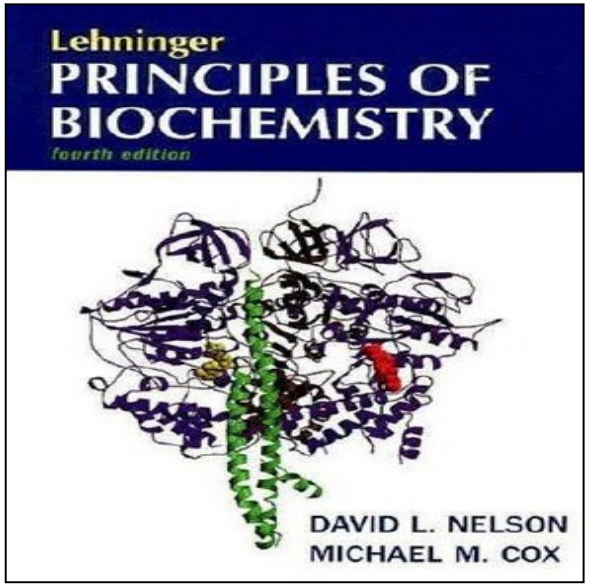
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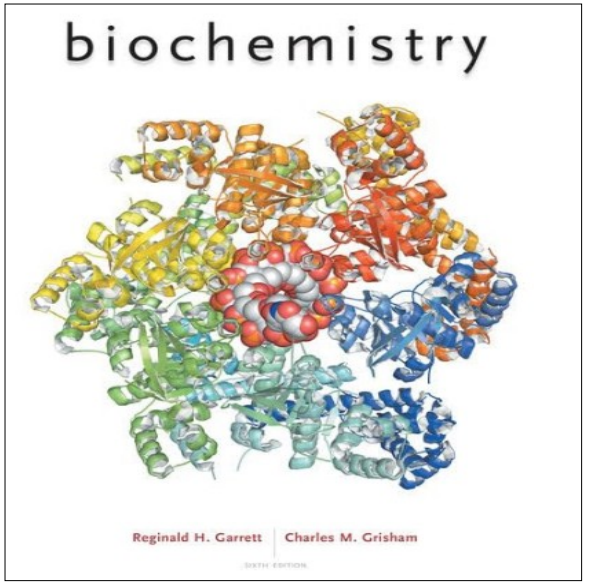
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